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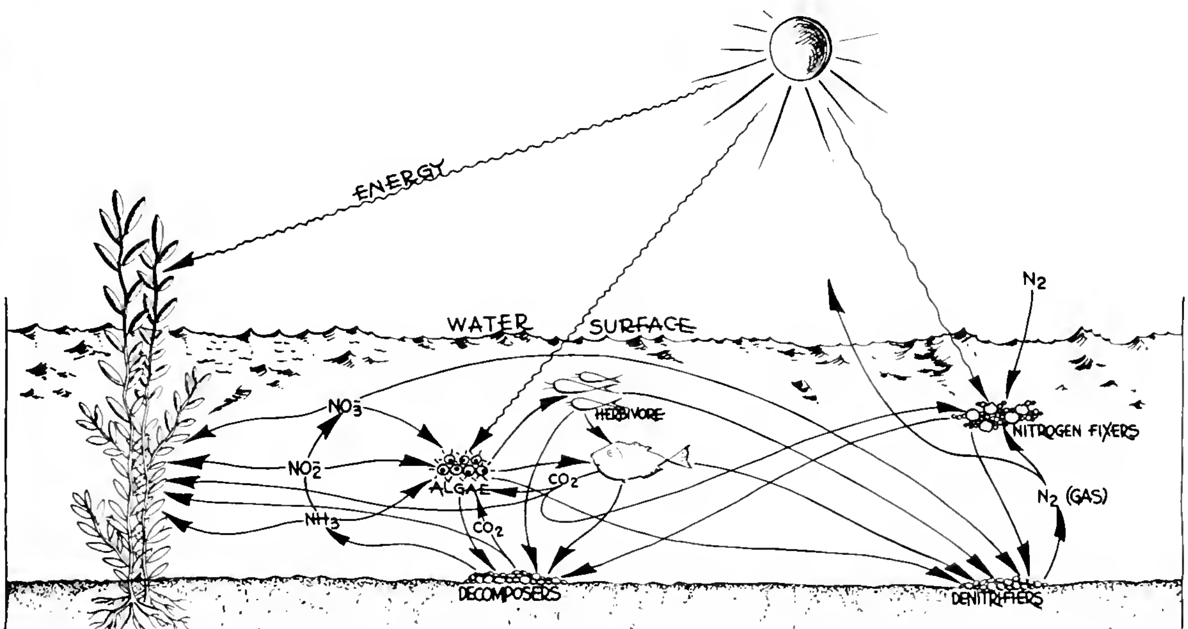
REMOVAL OF NITRATE FROM AGRICULTURAL TILE DRAINAGE BY A SYMBIOTIC PROCESS

BIO-ENGINEERING ASPECTS OF AGRICULTURAL DRAINAGE-SAN JOAQUIN VALLEY, CALIF.



SYMBIOTIC CYCLE

MAY 1976

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REMOVAL OF NITRATE FROM AGRICULTURAL TILE DRAINAGE BY A SYMBIOTIC PROCESS
May 1976

BIO-ENGINEERING ASPECTS OF AGRICULTURAL DRAINAGE–SAN JOAQUIN VALLEY, CALIFORNIA

A cooperative study by the
INTERAGENCY NITROGEN REMOVAL GROUP

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State of California

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ABSTRACT

A symbiotic bacterial-photosynthetic process was evaluated as a possible means of removing nitrate-nitrogen from subsurface agricultural drainage in the San Joaquin Valley of California. Three different versions, called systems, of the process were investigated. In one system the photosynthetic partner to the bacteria was algae, and in another, it was emersed grass. The third system was a combination of the first two. The study was directed toward general understanding of the process, assessment of seasonal effects on the process, development of preliminary costs for a treatment plant using the process, and an evaluation of the relative merits of the three systems studied.

The studies demonstrated that the symbiotic process could remove from 14 to 18 mg/l nitrogen from water initially containing 20 mg/l nitrate-nitrogen. Pond loadings to arrive at the 2-6 mg/l nitrogen effluent varied seasonally, with maximum and minimum loading rates in the summer and winter, respectively.

The grass system, with an estimated cost of $29 per million gallons of untreated water, was the least costly. The algal system followed by a harvesting process was estimated to cost $81 per million gallons of untreated water. A combination system in which the algal harvesting was replaced with a grass system reduced the estimated cost of the algal system to $38 per million gallons untreated water.

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SYMBOLS AND METRIC CONVERSIONS

	Symbol or abbreviation	Metric conversion
acre	AC	0.405 hectares
acre-feet	AF	1.234 cubic meters
add	+	
centimeter	cm	
day	d	
degrees Centigrade	°C	
degrees Fahrenheit	°F	
dollars	$	
equals	=	
feet	ft	0.305 meters
gallon	gal or g	3.785 liters
gallons per minute	gal/min or g/m	
gram	gm	
horsepower	hp	
hour	hr	
inch	in	2.540 centimeters
langleys		gram calorie per square centimeter
mile		1.609 kilometers
milligram	mg	
milligram per liter	mg/l	
milligram per square foot per day	$mg/ft^2/day$	
milliliter	ml	
million gallons	Mgal	3,785 kiloliters
minute	min or m	
most probable number	MPN	
multiply	*	
per	/	
pounds	lb	0.454 kilograms
square feet	ft^2	0.093 square meters

SUMMARY

Use of a symbiotic or denitrifying bacteria-photosynthetic plant process to reduce the nitrate-nitrogen content of subsurface agricultural drainage water in the San Joaquin Valley of California was evaluated during a 2-year study. The study was designed to define the nitrogen removal process, interpret the roles of the various mechanisms involved, assess the efficiency for removal of nitrogen under seasonal environmental conditions, and determine the relative costs of the systems.

Three versions of the symbiotic process were tested: (1) algae-bacteria in which the algae were grown in shallow outdoor cultures, providing the organic carbon source for denitrifying bacteria; (2) grass-bacteria in which water-tolerant grasses growing in shallow flooded ponds provided the organic carbon; and (3) a combination of algae-grass-bacteria in which algal ponds are followed by grass plots, eliminating the need for additional algal harvesting.

Removal Efficiency

The efficiency of the symbiotic process was evaluated to determine the rate at which nitrogen can be removed from the water and the concentration of dissolved nitrogen remaining in the water after treatment. The study was conducted with water containing nearly 20 mg/l nitrogen, of which 90 percent or more was in the nitrate form.

The algal-bacterial system was able to reduce total dissolved nitrogen to 5 mg/l during December and January and to 3 mg/l during the remaining months of the year. A combination of average daily air temperature of less than 10°C and sunlight less than 200 langleys per day appeared to be factors limiting effluent minimum dissolved nitrogen to 5 mg/l. Nitrate nitrogen was reduced to less than 1 mg/l in the most efficient ponds, except for the two winter months when it was 2 to 3 mg/l. Biological activity in the ponds produced nitrite, and occasionally ammonia, and increased soluble organic nitrogen. Ammonia concentrations near one-half a milligram per liter were associated with water temperatures near 32°C or rainfall during the winter. Nitrite was commonly found in concentrations of 0.05 mg/l-N or less, but reached 0.5 mg/l during the summer months.

Soluble organic nitrogen was the largest dissolved nitrogen fraction found in algal pond effluent in all but the two winter months of December and January. It averaged 2.5 mg/l during the

summer months, 2.0 mg/l during the spring and fall months, and about 1.7 mg/l during the winter.

The grass-bacterial system reduced total nitrogen to between 2 and 5 mg/l, averaging near 3 mg/l annually. About 2 mg/l of this 3-mg/l average was organic nitrogen and the rest mostly nitrate-nitrogen. Effluent nitrite-nitrogen concentrations were negligible.

During the months of April through September, nitrogen mass removal rates varied from 30 to 50 $mg/ft^2/day$ for the algal ponds and 50 to 75 $mg/ft^2/day$ for the grass ponds. From October through March, these removal rates decreased respectively to 15 to 30 and 20 to 50 $mg/ft^2/day$.

The soluble organic nitrogen produced by the biological activity in the ponds was the limiting factor in reducing total dissolved nitrogen to less than 3 mg/l in the effluent. The soluble organic nitrogen averaged 1.5 to 2.5 mg/l in the pond effluent for all the systems.

The soluble organic nitrogen studies at Firebaugh and Stanford University (Parkin and McCarty, 1973) showed that within 30 days, 30 to 50 percent of the soluble organic nitrogen in pond effluent was regenerated and biostimulatory. The remaining 70 to 50 percent was not available for algal assimilation and showed little decomposition for up to 120 days.

In the algal-bacterial system, algal cellular nitrogen (total particulate nitrogen) in the effluent was generally about 3 mg/l-N and part of this was potentially biostimulatory upon the death and decay of the algal cells. Harvesting of two-thirds of this biomass would usually reduce cellular nitrogen in the effluent to 1.0 mg/l. Foree and McCarty (1968) and Jewell and McCarty (October 1971) have shown 30 to 60 percent of the nitrogen in algal cells is not regenerated and is resistant to long-term biodegradation. Thus biostimulatory nitrogen in algae cells remaining after harvesting should be less than 0.5 mg/l.

After treatment the subsurface drainage effluent collected from the drains beneath the grass ponds was the lowest in nitrogen concentration. In an area where percolating water can be recovered, maximizing percolation and mixing the recaptured subsurface drainage with surface effluent would yield an effluent with the least nitrogen.

Summary

The Removal Process

In the algal-bacterial system, anaerobic denitrification apparently removed an average of 50 percent of the influent nitrogen. An average of 30 to 35 percent of the influent nitrogen was incorporated into algal cells and 15 to 20 percent left the pond via the effluent as dissolved nitrogen. Of the 30 to 35 percent incorporated into the algal cells, half accumulated in the pond sludge and the other half was in the effluent prior to algal harvesting.

In the grass ponds, anaerobic denitrification can remove 85 to 90 percent of the influent nitrogen. Grass and algal assimilation of nitrogen was considered to be minimal. Removal rates for the reed canarygrass pond were greater than those for the watergrass or sprangletop ponds except for January through May.

Sunlight and temperature appeared to be the most important physical environmental factors controlling the rates at which nitrogen was removed from the waste water. Loading rate and detention time were the most important controllable factors affecting nitrogen removal.

Algae and grass were found to be readily available organic carbon sources for anaerobic denitrifying bacteria. Algal types present were mostly unicellular flagellate and nonmotile green algae. Algal population varied seasonally, with highs in the spring and fall and lows in the summer and winter.

Effect of salinity on grass varieties produced varying response to survival. At salinities (total dissolved solids) as high as 15,000 mg/l, there are plant varieties that in a flooded condition will produce several tons per acre of organic matter per year. According to Williford and Cardon (1971), 1 ton of organic matter provides adequate energy for the complete removal of nitrate-nitrogen from 6 acre-feet of water containing 20 mg/l of nitrogen in the nitrate form with 8 mg/l of dissolved oxygen. As the anticipated total annual yield of the San Luis Drain is 150,000 acre-feet (approximately 49,000 million gallons), at the above concentration the total organic matter requirement is approximately 25,000 tons annually. Average production (tons per acre), at salinities near 6,800 mg/l TDS, that could be expected from the several grasses for which production records are available would be sprangletop 4.4, watergrass 4.7, alkali bulrush 5.4, and reed canarygrass 11.2 tons per acre.

Denitrification by bacteria residing near the soil-water interface appeared to be the most important nitrogen removal pathway in the symbiotic process. Measurable (test was not definitive)

populations of denitrifying bacteria were between 10^5 and 10^6 MPN/cm^2 in the water-sludge-soil profile column. The water alone had measurable populations in the 0 to 1,000 cells per milliliter range.

In studies comparing soil-lined ponds to fiberglass-lined ponds, the soil immediately provided a favorable environment for the denitrifying bacteria. In contrast, the environment in the nonsoil ponds was not very favorable until a sludge layer had developed. This was evident in the rates of removal which were lesser in the nonsoil ponds than in comparable soil-lined ponds during the initial operating period.

In the grass ponds after a year's operation, most of the nitrogen assimilated into plant material remained in organic matter on the soil surface. In the algal ponds, from 150 to 400 pounds of nitrogen per acre per year were added to the sludge. With a sludge dry weight nitrogen content of 1 percent, the accumulation would amount to 15 tons of dry organic material per acre per year.

Process Evaluation

With 20 mg/l nitrate-nitrogen as the influent concentration, the following results can be expected from three versions of the symbiotic process to annually treat 150,000 acre-feet (49,000 million gallons) of subsurface agricultural drainage:

System	Effluent total nitrogen (mg/l)	Estimated cost dollars/million gallons (1972 dollars)	Treatment area required (acres)
Algae-bacteria	4-6	81	8,600
Grass-bacteria	2-5	29	6,500
Combination	2-5	38	10,000

Algal harvesting by chemical separation was investigated in jar tests. The investigation demonstrated that 40 mg/l $Fe_2(SO_4)_3$ would flocculate and settle 67 percent of the algae cells in the effluent. The remaining 33 percent of the cells would contain approximately 1 mg/l nitrogen. In a soluble organic nitrogen study, Parkin and McCarty (1973) found iron salts at 50 p/m (parts per million) would remove 39 percent of the soluble organic nitrogen in filtered pond effluent.

The summertime nitrogen removal rates in algal ponds of 1-, 3-, or 6-foot depths were similar when the ponds were compared on the

basis of loading-per-unit of surface area. Grass ponds were effective in nitrogen removal at depths up to 0.8 foot.

It was necessary to add phosphorus to the algal test ponds to promote the necessary amount of algal growth.

Because watergrass does not withstand well the aggressive nature of bulrushes, grass ponds would need renovation every 3 to 5 years.

Annual operation and maintenance must include control of muskrats. These rodents will invade the ponds and by burrowing through the dikes could effectively short-circuit the ponds.

Conclusions

1. Subsurface tile drainage water, with phosphorus augmentation for algal growth, will support adequate growth of algae or grass to facilitate removal of 14 to 18 mg/l nitrogen from an initial concentration of 20 mg/l nitrate-nitrogen using the symbiotic process.

2. Although none of the systems studied appear to be capable of achieving 2 mg/l total effluent nitrogen on a sustained basis, available data indicate that the 2 mg/l total nitrogen objective may be unrealistic in terms of the ability of various effluent nitrogen forms to stimulate algal growth in potential receiving waters. A portion (median of 60 percent) of the dissolved organic nitrogen in the effluent is resistant to degradation for up to 120 days.

3. Comparing the data in this report with data gathered during earlier studies of algae stripping and bacterial denitrification, it appears that the symbiotic process can produce similar quality effluents at substantially lower costs.

4. The grass-bacteria and the combination systems appear to be most worthy of further consideration in terms of both cost and removal efficiency.

Recommendations

Investigations of methods for disposal of agricultural waste water in the San Joaquin Valley should consider additional beneficial uses for the untreated waste water, the nitrogen removal treatment ponds, and the denitrified waste water to reduce the cost of the waste-water disposal. Possible beneficial uses are:

Summary

1. Process cooling water such as for nuclear generators.

2. Food production in marine or brackish water environments.

3. Development of sport fish and wildlife areas.

4. If nitrogen removal is found necessary, marketing a portion of the algae or grass harvest for a protein source, food source, or soil amendment.

The criteria to be used in assessing treatment process alternatives to remove nitrate-nitrogen from the San Joaquin Valley drainage should be based on the reliability of the alternative, multiple-use benefits, discharge criteria, availability of land, byproduct markets, and the discharge location. This assessment should be summarized in a report, and large-scale pilot studies, for operating and design criteria for the best alternative, should be initiated prior to the need to discharge the San Luis Drain to the estuary.

The soluble organic nitrogen studies have demonstrated that 50 to 70 percent of the soluble organic nitrogen in the pond effluent was not available for algal assimilation and showed little decomposition for a period up to 120 days. This resistant amount, 0.5 to 1.0 mg/l of nitrogen, should be petitioned for exclusion from waste discharge requirements.

INTRODUCTION

This is the final report of field research on the feasibility of using a combination (bacterial-photosynthetic plant) process, the "Symbiotic Process," as a method of removing nitrate-nitrogen from subsurface agricultural drainage in the San Joaquin Valley. The symbiotic studies were conducted by members of the Interagency Nitrogen Removal Group at the Interagency Agricultural Wastewater Treatment Center (IAWTC) located near Firebaugh, California (figure 1). Field work began in July 1971 and continued through October 1973.

Purpose and Scope of Report

This report presents the findings of an intensive interagency investigation of a practical means to control the nitrate concentration in subsurface agricultural waste water prior to its discharge into a receiving water body. The investigation deals with two different photosynthetic organisms, grass and algae, in partnership with bacterial organisms.

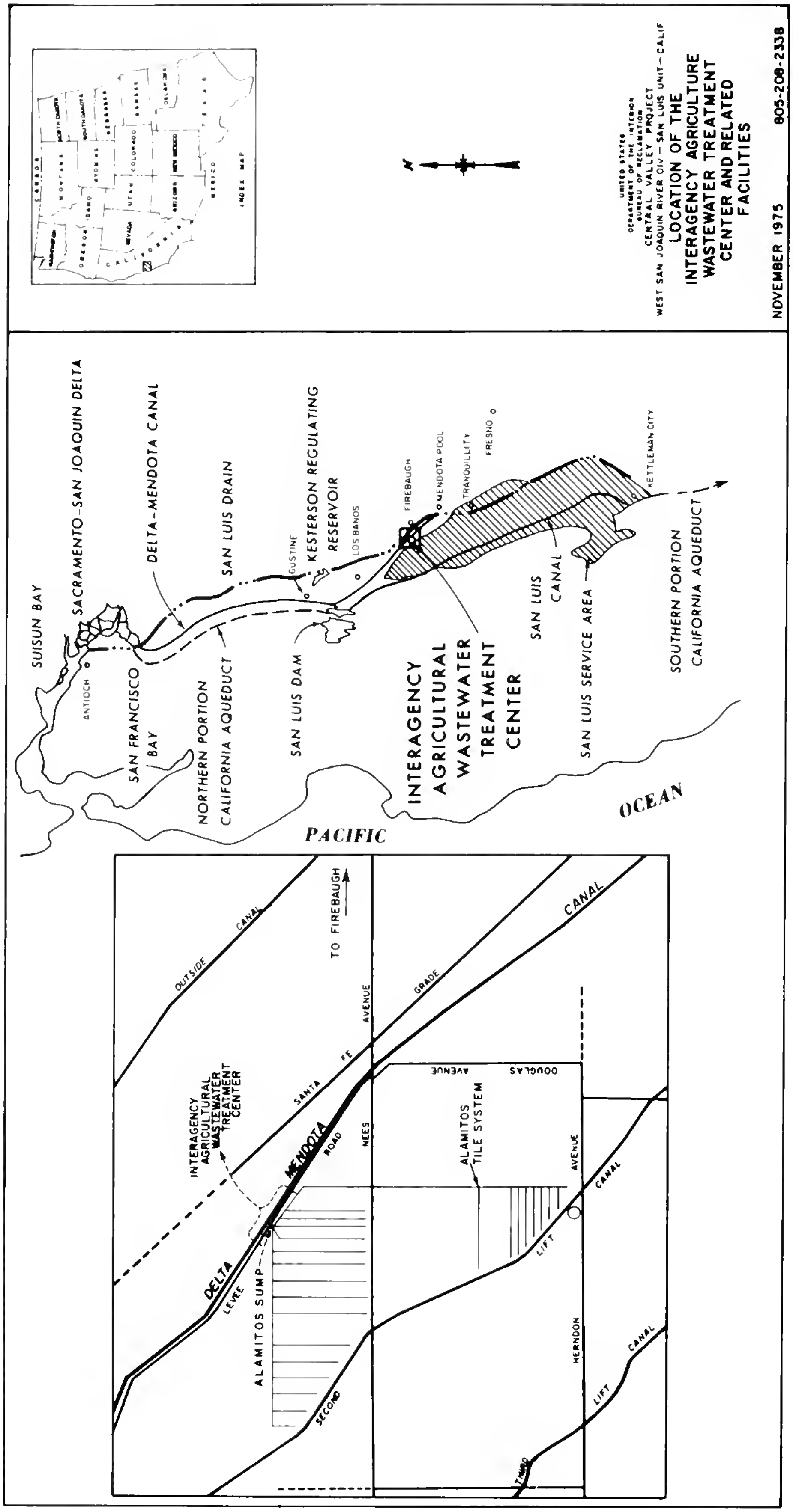
Previous Investigations

The ultimate need to provide subsurface drainage collection for large areas of agricultural land in the western and southern San Joaquin Valley has been recognized for some time. In 1954 the Bureau of Reclamation feasibility report for the San Luis Unit contained a plan to provide drainage collection for the service area including an export facility. In 1957 the California Department of Water Resources initiated an investigation to assess the extent of salinity and high ground-water problems and to develop plans for drainage. The Burns-Porter Act in 1960 authorized San Joaquin Valley drainage facilities as part of the State Water Facilities.

The San Luis Unit authorizing legislation (Public Law 86-488), passed in June 1960, included drainage facilities as a part of the project features. This Act requires the Secretary of the Interior to construct the San Luis Drain to the Delta or to insure that the State of California would provide a master drain for the San Joaquin Valley that would adequately serve the San Luis Unit.

Investigations by the Bureau of Reclamation and the Department of Water Resources revealed that serious drainage problems already exist and that areas requiring subsurface drainage would probably exceed 1 million acres by the year 2020. Disposal of the drainage waters into the Sacramento-San Joaquin Delta near Antioch, California, was found to be the least costly plan alternative.

Figure 1


SUISUN BAY
SACRAMENTO-SAN JOAQUIN DELTA
DELTA-MENDOTA CANAL
SAN LUIS DRAIN
KESTERSON REGULATING RESERVOIR
GUSTINE
LOS BANOS
FIREBAUGH
MENDOTA POOL
TRANQUILLITY
FRESNO
KETTLEMAN CITY
ANTIOCH
SAN FRANCISCO BAY
NORTHERN PORTION CALIFORNIA AQUEDUCT
SAN LUIS DAM
INTERAGENCY AGRICULTURAL WASTEWATER TREATMENT CENTER
SAN LUIS CANAL
SAN LUIS SERVICE AREA
SOUTHERN PORTION CALIFORNIA AQUEDUCT
PACIFIC
OCEAN
INDEX MAP
N
UNITED STATES
DEPARTMENT OF THE INTERIOR
BUREAU OF RECLAMATION
CENTRAL VALLEY PROJECT
WEST SAN JOAQUIN RIVER DIV – SAN LUIS UNIT – CALIF
LOCATION OF THE
INTERAGENCY AGRICULTURE
WASTEWATER TREATMENT
CENTER AND RELATED
FACILITIES
NOVEMBER 1975
805-208-2338
OUTSIDE CANAL
TO FIREBAUGH
AVENUE
GRADE
CANAL
SANTA FE
NEES
DOUGLAS AVENUE
ALAMITOS TILE SYSTEM
INTERAGENCY AGRICULTURAL WASTEWATER TREATMENT CENTER
DELTA
MENDOTA
ROAD
LEVEE
ALAMITOS SUMP
SECOND
LIFT CANAL
HERNDON AVENUE
THIRD

Introduction

Preliminary studies indicated the drainage water would be relatively high in nitrogen. The then Federal Water Pollution Control Administration conducted a study to determine the effect of discharging such drainage water on the quality of water in the San Francisco Bay and Delta. Based on the results of this study, completed in 1967, it was concluded that at certain concentrations the nitrogen content of untreated drainage waters could have significant adverse effects upon the fish and recreation values of the receiving waters. A 3-year research program to determine the economic feasibility of nitrate-nitrogen removal was recommended.

As a consequence, the Interagency Agricultural Wastewater Study Group (U.S. Bureau of Reclamation, California State Department of Water Resources, and Environmental Protection Agency) completed a 4-year cooperative research program. Elements of that research included an inventory of nitrogen conditions in the potential drainage areas, possible control of nitrates at the source, prediction of drainage quality, changes in nitrogen in transit, and methods of nitrogen removal from drain waters including biological-chemical processes and desalination.

These previous studies into methods of nitrogen removal had demonstrated technically feasible methods (Brown, 1971a); however, the treatment costs estimated from these studies were higher than expected. After a review of the data collected in the earlier algal growth and harvesting studies, it was concluded that a combined system involving bacteria and algae warranted further study. In the preliminary studies of the symbiotic process, the results suggested that mechanical equipment costs could be reduced by eliminating pond mixing while equivalent performance in nitrogen removal would be retained (Arthur, 1971; Brown, 1971b).

In another study of nitrogen removal from agricultural waste water, Williford and Cardon (1971) reported that flooded grass plots provided an effective nitrogen removal system. The removal mechanism appeared to be similar to that found in the algae-bacteria system. Preliminary cost estimates indicated that this treatment process was also an economical alternative deserving further study.

Based on the above information, the U.S. Bureau of Reclamation (USBR) and the California Department of Water Resources (DWR) continued studies of the symbiotic process in both the algae-bacteria and grass-bacteria configurations.

Introduction

Process Description

The symbiotic process consists of a combination of oxic (molecular oxygen required) and anoxic (no molecular oxygen required) reactions involving bacteria and photosynthetic plants. Nitrate-nitrogen enters a pond and a portion is assimilated by the growing algae or grass into cellular organic nitrogen. As the algae or grass die they settle to the bottom where, under anoxic conditions, they are decomposed by denitrifying bacteria, which use another portion of the dissolved nitrate for oxidation of the organic material. In the process the nitrate is reduced to nitrogen gas.

As the plants are decomposed by bacteria, the cellular organic nitrogen is released as ammonia, peptide chains, amino acids, and other nitrogen-containing compounds. Most of the ammonia is reassimilated by growing algae and grasses and the cycle is continued. The nitrogen in the pond effluent thus consists of the unused nitrate-nitrogen, nitrogen incorporated into plant cells, the nitrogen-containing decomposition products, and perhaps a small amount of ammonia. The effluent contains less inorganic combined nitrogen than the influent because some is lost as nitrogen gas (N_2) and because some plant cells (and their nitrogen) settle and accumulate on the bottom of the pond. Additional nitrogen can be removed if any particulate nitrogen (algal cells, for example) is harvested.

The theories explaining the growth of the organisms contributing to the above system have been described elsewhere (Brown, 1971a; Sword, 1971; and Williford and Cardon, 1971).

Purpose of the Study

The general research objectives of the studies were to:

1. Define the subsystems operating in the removal process and interpret the roles of the soil, grass, algae, bacteria, and waste water in removing nitrogen.

2. Assess the efficiency of the process for removing nitrogen (nitrate and total) under varying seasonal environmental conditions.

3. Develop unit design criteria and operating criteria for a process capable of achieving various levels of effluent nitrogen.

4. Assess the relative merits of the two symbiotic process systems.

The information from these studies is expected to help solve the specific problem of nitrogen removal from agricultural waste water in the San Joaquin Valley of California, and also to provide general information concerning nitrogen removal from nitrate-enriched waters in other localities.

Acknowledgments

The primary participants in the investigation were the United States Bureau of Reclamation and the California Department of Water Resources. Other agencies who cooperated to a lesser extent were the Environmental Protection Agency and the California State Water Resources Control Board. The two primary agencies initiated the program because they are responsible for providing a system for disposing of subsurface agricultural waste water from the San Joaquin Valley of California and protecting water quality in California's water bodies. Others cooperated in the program by providing specialized or specific knowledge pertinent to the research undertaking.

This report was reviewed by the United States Bureau of Reclamation, the California Department of Water Resources, and technical consultants Robert C. Cooper, Ph.D., and Perry L. McCarty, Sc.D.. Randall Brown of the California Department of Water Resources edited the report.

MATERIALS AND METHODS

The Interagency Agricultural Wastewater Treatment Center (IAWTC), which includes a chemical-biological laboratory, is located on the right-of-way of the Delta-Mendota Canal (figure 1). Study ponds for both the grass system and the algae system were located at the Center; additional grass study ponds were located on privately owned land, "the Bennett plot," about 13 miles west-northwest of the Center.

The IAWTC was staffed by personnel from the California State Department of Water Resources and the United States Bureau of Reclamation. An interagency coordinating committee supervised the performance of the studies while technical guidance was provided by a panel of consultants consisting of: Robert C. Cooper, Ph. D., Professor of Environmental Health Science of the University of California, Berkeley; Perry L. McCarty, Sc. D., Professor of Environmental Engineering, Stanford University, Stanford; A. Douglas McLaren, Ph. D., Professor of Biology, University of California, Berkeley.

Irrigation return water for the Center was obtained from the Alamitos tile drainage system. The quality of the water has been documented by Brown (1971a). Results of two more recent mineral analyses are listed in table 1. During low winter flows, the tile system was inadequate for the demands of the Center. During these periods, the water supply was augmented by water from a Bureau of Reclamation ground-water interceptor drain which runs through the Center and parallel to the Delta-Mendota Canal.

Table 1. Water quality analyses of the Alamitos tile drain water (milligrams per liter)

Constituent	Sample date 3/17/70	Sample date 9/14/70
Sodium	1,360	669
Calcium	370	141
Magnesium	184	86
Potassium	10	3.2
Boron	15	6.4
Sulfate	3,270	1,410
Chloride	545	233
Nitrate-Nitrogen	26.7	12.7
Bicarbonate (as $CaCO_3$)	350	376
Carbonate (as $CaCO_3$)	0	0
Hardness	1,680	707
Total dissolved solids	6,370	2,940
Specific conductance (micromhos)	7,450	3,780

Materials and Methods

The specific conductance of the interceptor drain water averaged 8,500 micromhos per centimeter and the nitrate-N content averaged 8 mg/l.

The supply water was stored in an 820,000-gallon covered and plastic-lined storage pond at the Center. The water's nutrient content was supplemented by the addition of sodium nitrate and phosphoric acid. Sodium nitrate was added to make the nitrate content of the water supply more nearly representative of the average level of 20 mg/l nitrate-nitrogen projected for the San Luis Drain. Phosphoric acid was added to promote algal growth, as earlier studies had demonstrated that phosphorus was limiting to algal growth in tile drainage (Brown, 1971a). The previous studies at the Center indicated a ratio of 1 mg/l phosphorus to 10 mg/l nitrogen was necessary for maximum algal growth. Long-term operation of the symbiotic algal ponds showed that 100 mg/l was the average algal biomass concentration. The algal biomass had a dry weight nitrogen content of 10 percent. Using these facts as a basis, the supply water was augmented with 1 mg/l phosphorus. (From the long-term operational unit's performance, it was assumed that 50 percent of the influent nitrogen would be denitrified.) Table 2 shows the supply water quality after phosphorus and nitrogen had been added. The supply water was pumped from the storage pond into a pipeline supplying all the study ponds at the Center.

Table 2. Water quality of treatment center modified supply after N and P additions (milligrams per liter)

Constituent	July 1971 to November 1972	
	Mean	Standard deviation
Nitrogen		
Ammonia	0.01	0.02
Nitrate	18.2	2.7
Nitrite	0.11	0.18
Total inorganic	18.3	2.7
Dissolved organic	0.37	0.19
Total organic	0.41	0.25
Particulate	0.04	0.12
Dissolved	18.7	2.6
Total	18.8	2.5
Orthophosphate - P	1.13	0.41
Water temperature - (°C)	18.3	4.5
Bicarbonate alkalinity (as $CaCO_3$)	313	42
pH (units)	7.66	0.14
Total suspended solids	27.7	9.6
Volatile suspended solids	9.0	6.6

The Bennett plot water supply was obtained from a drainage ditch of the Panoche Drainage District. The nitrate content of this water was also supplemented with sodium nitrate to reach a level of approximately 20 mg/l nitrogen. Table 3 contains some chemical data for the supply water after the nitrogen content had been increased. The influent nitrogen concentrations at the Bennett plot did not remain as steady as those at the Center because of the variety of waters contributing to the drainage ditch and because storage facilities to provide a more uniform blend of supply water were lacking.

Table 3. Chemical characteristics of the modified water supply for the Bennett plot (milligrams per liter)

Constituent	July 1971 to November 1972		
	Minimum	Mean	Maximum
Nitrogen			
Ammonia	0.0	0.07	0.8
Nitrate	1.0	18.4	32.0
Nitrite	0.1	0.3	1.8
Total inorganic	3.0	18.9	32.1
Dissolved organic	0.4	0.9	1.1
Total organic	0.4	1.4	3.3
Particulate	0.0	0.6	1.1
Total dissolved	3.5	19.5	32.5
Total	4.0	19.7	33.0
Orthophosphate - P	0.1	0.15	0.2
Total suspended solids	30	121	320
Volatile suspended solids	1	17	52
Specific conductance (micromhos)	2,200	5,200	10,500
pH (standard units)	7.5	8.1	8.4
Water temperature (°C)	0.0		34.0

Algae-Bacteria Symbiotic Process

The algae-bacteria symbiotic process was studied in small to moderate sized ponds at culture depths of 1, 3, and 6 feet. Table 4 lists the ponds used as well as some of their characteristics.

Table 4. Operational units for the algae-bacteria symbiotic process

Unit No.	Depth (feet)	Bottom cover	Startup date
1	1.0	Soil	7/71
5	1.0	Soil	7/71
6	1.0	Soil	7/71
7	1.0	Soil	7/71
8	1.0	Soil	7/71
11	1.0	No-soil	7/70
12	1.0	No-soil	3/70
16	0.3	Soil-grass	5/72
17	1.0	No-soil	7/71
18	1.0	No-soil	7/71
19	1.0	No-soil	8/71
20	1.0	No-soil	8/71
21	1.0	Soil	8/68
22	1.0	Soil	8/68
301	3.0	Soil	8/71
302	3.0	Soil	8/71
601	6.0	Soil	8/71
602	6.0	Soil	8/71

The 14 resin-coated plywood ponds used to study nitrogen removal at the 1-foot culture depth (figure 2) were some of the "miniponds" described by Brown (1971a). Of these 14 ponds, 13 were 16 feet long by 8 feet wide with a water surface area of 128 square feet. In the ponds the nominal water depth, or in some cases, the water plus soil depth was 1 foot. The initial 1- to 2-inch soil lining for seven of the 13 ponds came from soil collected from around the site.

Of these 13 ponds, two soil-lined and two unlined were continued from the previous studies and had produced the preliminary data on the symbiotic algal system. These four ponds had a length-to-width ratio of 2. The other nine ponds had a center baffle extending lengthwise from one end to within 2 feet of the opposite end, creating folded ponds with a length-to-width ratio of 8. A chloride tracer flow study to characterize the distribution of a discrete portion of the influent water mass with time through the pond indicated 73 percent plug flow (tracer mass remained intact) and 27 percent mixed flow (tracer mass mixed with other water) with no stagnant areas in these nine ponds. This indicates that the majority of the water mass passed through the ponds at near the computed detention times. These were the only ponds characterized as to flow.

The fourteenth pond was 16 feet long by 4 feet wide with a water surface area of 64 square feet. The pond had 6 inches of soil placed

over the bottom, with alkali bulrush and watergrass planted in the soil and ponded with approximately 3 inches of water. The effluent from an algal pond was the water supply for this grass pond. The grass pond was used to test the ability of the grass to remove the suspended algae from the water as an alternative algal separation process. The separation process can be described as grass straining the algae from the water.

Figure 2. Algal ponds - 1 foot in depth

The 3-foot-depth ponds (figure 3) were made by bisecting a large 1/4-acre algal pond used in the previous studies. Each of the resulting ponds was approximately 95 feet long by 54 feet wide with a surface area of 5,100 square feet. A lengthwise center baffle produced an effective length of the ponds of 190 feet and a width of 27 feet, giving a length-to-width ratio of 7. Two to 4 inches of soil were placed on the asphalt lining to provide a soil environment on the bottom.

Figure 3. Algal pond - 3 feet in depth

The 6-foot-depth ponds were also made by modifying ponds from the previous studies (figure 4) In one case, a 12-foot-depth digestion pond which had received effluent from the 1/4-acre algal growth pond was filled in to provide the proper depth. Prior to the filling operation, accumulated sludge in the pond was removed to eliminate any influence this might have on pond efficiency. These 6-foot-depth ponds were approximately 72 feet by 72 feet with a surface area of about 5,200 square feet. A vinyl plastic center baffle created folded ponds with an effective length of 145 feet and a width of 36 feet for a length-to-width ratio of 4. These ponds did not have an impervious lining.

The flow rate of water into the 1-foot-depth ponds was measured by glass-tube rotameters, and into the 3- and 6-foot ponds by recording flow meters. Because it was difficult to maintain steady flow conditions, the flow rates into all ponds were read and recorded daily and reset to the desired flows. Average flows were then calculated by taking the mean of the beginning and ending flow rates for each time period.

Figure 4. Algal pond - 6 feet in depth

The effluent flow rates of the ponds could not be conveniently measured so they were calculated by subtracting the net evaporation rate from the influent flow rate. Percolation from the ponds was assumed to be zero because all of the ponds had an impervious lining except the 6-foot-deep ponds which were unlined.

The algal ponds were operated at flow rates designed to bracket detention times found to be satisfactory in previous studies. Ponds of different depths were compared by operating at least one pond at each depth at the same influent nitrogen loading per unit surface area.

Two methods of sampling were used on the algal system. The most common method was to collect a single grab or dip sample at the effluent once per sampling period. The second method used on some ponds consisted of battery-powered composite samplers which took 60-ml samples every 30 minutes. These compositors usually collected the composite sample over a 1-day period from the pond effluent.

Materials and Methods

A system of sampling the sludge in the 1-foot-depth ponds was developed to measure the accumulation of carbon, nitrogen and phosphorus on the pond bottom. Ponds were divided into 2-foot-square grid sections and sediment core samples were collected from 11 to 12 sections selected at random. Sample cores were made by pushing a 1.0-cm-diameter glass tube into the sludge to the pond bottom. Four cores were collected from each selected grid section. The depth of the sludge-soil profile was measured in each core to determine average sludge depth and then all of the subsamples were combined into one sample.

Grass-Bacteria Symbiotic Process

Shallow grass pond studies were carried out on five small rectangular ponds 10 feet by 50 feet at the Center and on two large ponds (3.5 and 3.9 acres) at the Bennett plot (figures 5 and 6). A sixth small pond at the Center was covered to prevent the growth of grass and served as a control pond. Table 5 lists the operational unit for the grass symbiotic system.

Table 5. Operational units for the grass-bacteria symbiotic process

Unit	Depth (feet)	Grass type	Startup date*
GP 1	0.6	Watergrass-alkali bulrush	7/72
GP 2	0.3 to 0.6	Watergrass-alkali bulrush	7/72
GP 3	0.5 to 0.7	Watergrass-alkali bulrush	7/72
GP 4	0.3	Watergrass-alkali bulrush	
GP 5	0.3 to 0.6	Reed canarygrass	3/72
GP 6	0.75	None	1/72
Bennett East	0.5	Sprangletop-watergrass	7/72
Bennett West	0.25	Watergrass-sprangletop	7/72

*Most ponds were started in late 1971 but effective data collection began on the date indicated.

To test the effect of salinity levels on the growth of various species of grass, 54 microplots (4 x 4 feet) of land were used. In these microplots, waters of various salinities (approximately 5,000, 10,000, and 15,000 mg/l total dissolved solids) were tested on different kinds of grass to determine their effect on plant growth.

Two species of grass (with the exception of the microplots) were chosen for investigation in the pond studies. Watergrass (*Echinochloa crusgalli*) was the main grass variety studied while reed canarygrass

Figure 5. Small rectangular grass ponds

Figure 6. Large grass ponds at the Bennett plot

(*Phalaris arundinacea*) was investigated in one small pond. In one of the larger ponds, there was a succession from the watergrass to sprangletop. Watergrass or wild millet is an annual of the seed plant group which grows best in moist soil and mudflats, and will tolerate moderately saline soil. Watergrass can reach maturity in 60 to 80 days and will stand considerable flooding after the plants are established. The growing season is relatively short due to late spring germination and early winter kill. Once a pond is seeded, it will continue to reseed itself, but management is necessary to prevent crowding out by other undesirable plants (California Department of Fish and Game, 1960).

Reed canarygrass is a perennial grass of the seed plant group which can survive in continuous flooded conditions. The grass is well adapted to all medium-textured to fine-textured soils and moderately well adapted to alkaline and saline soils. The native environment of this grass is in heavy wet soils, but it will do well on light-textured soils with ample irrigation. Hot weather will cause dormancy. Growth is also slowed by cool weather; thus the greatest periods of growth are during the spring and fall seasons (Finch, 1972).

Bearded sprangletop (*Leptochloa fascicularis*), also known simply as sprangletop, is a summer annual grass (often a weed in rice fields). It is found in alkali flats, ditches, and marshes (Hitchcock, 1950). It is tolerant of waterlogging, high salinity, and high alkalinity. Its growth is similar to watergrass, but it produces less seed and is less attractive to waterfowl.

In the specific study of the effect of salinity on the growth of grass, additional grass species were used. These included alkali bulrush, goars fescue, and coastal and cross-coastal bermuda grass.

Alkali bulrush (*Scirpus robustus*) is a long lived perennial plant belonging to the sedge family. It commonly occurs in salt, fresh-water, and alkaline marshes. The plant seed is a leading duck food. Alkali bulrush is recommended for planting especially on sites where the soil is highly saline or alkaline. The plant normally propagates from seed (during germination it is sensitive to high salinity), but once established, it will spread rapidly by horizontal rhizomes (State of California, Department of Fish and Game, 1963).

Goars fescue (*Festuca arundinacea*) has the ability to produce well on wet, saline and/or alkali, fine-textured soils. It is a robust long lived perennial bunch grass with the ability to remain green during summer months although its best growth is in relatively

cool weather and will continue some growth when mean temperatures are as low as 34°F. The root system is extensive and tough (Phillips Petroleum Company, 1960).

Coastal bermuda grass and coast cross bermuda grass are varieties of Cynodon dactylon, a warm season creeping perennial, sod forming, turf grass which propagates by seed, runners, and underground rootstocks. It has extensive scaly rhizomes and grows best when mean temperatures exceed 75°F. When mean temperatures fall below 60°F, it produces very little growth. Temperatures near 27°F kill the stem. It is adapted to fine-textured soils and is tolerant of high salinity. It tolerates flooding for long periods but makes little growth on waterlogged soils. Coastal bermuda grass is taller, has a larger leaf, and produces less seed than the basic bermuda grass, but it is more frost resistant and grows better in cool weather than bermuda grass. Coast cross bermuda grass is coarser and more vigorous than coastal bermuda grass (Phillips Petroleum Company, 1960).

The small grass ponds were constructed by excavating soil to an approximate depth of 2.5 feet, laying a 6-mil polyethylene film liner, replacing about 1 foot of soil, installing subsurface drains, then replacing the remainder of the soil. Baffles were placed in the ponds to reduce short-circuiting of the surface water. The baffles gave the ponds an effective length of 70 feet with a length-to-width ratio of 7. These ponds were constructed in a slightly to moderately saline oxalis silty clay.

Four of the small ponds were planted with watergrass, one with reed canarygrass, and the control pond was covered with styrofoam planks to exclude light, thus preventing plant growth. The reed canarygrass was allowed to become established; then it was clipped before inundating the pond. The watergrass ponds were inundated soon after germination.

Water depths of 3 inches to 8 inches, controlled by the elevation of the surface outlet, were studied in the small grass ponds. Other controllable variables were the subsurface and influent flow rates.

The two large field-size ponds at the Bennett grass plot (figure 7) were constructed in moderately heavy, saline clays (soil type mapped as Lethent). The Bennett grass plot is underlain by four tile drains installed at approximately 6-foot depths and 200-foot spacings. The lines varied in length from 300 to 480 feet. In 1972, the outfalls were raised about 1.5 feet to submerge the tiles.

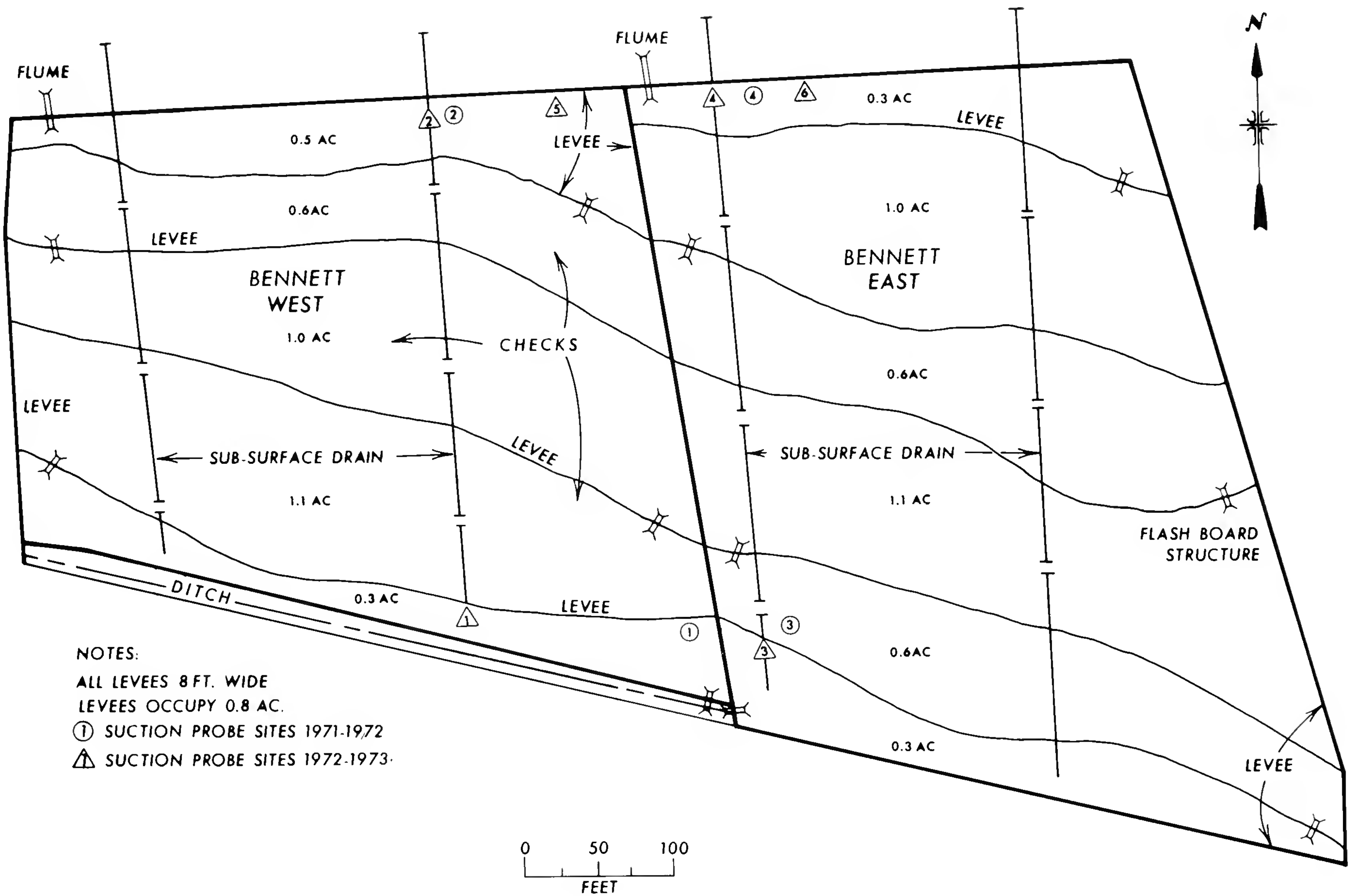


FIGURE 7

LOCATION DIAGRAM OF THE BENNETT GRASS PLOTS

Materials and Methods

At the beginning of the study in June 1971, the land was rough leveled, bordered, and levees built at the 0.3-foot-contour intervals. The individual ponds (checks) were divided by 1-foot-high earth levees. The water was passed through these levees by a wooden flash-board structure about 2.5 feet wide. Only one such structure was used per levee. The east pond, comprising a land area of 3.9 acres, was divided into six checks and the west pond, with a land area of 3.5 acres, into five checks. The west pond had a length-to-average-width ratio of 28, the east pond a ratio of 23.

Ground-water sampling sites were located in both of the large ponds. At these sites, suction probes, made out of ceramic cup filters and suction lines, were installed at various depths to 10 feet. At the main ground-water sampling sites, located near the subsurface tile drains, the probes were installed at 1-foot-depth intervals between 6 inches and 54 inches, with a final cup at 10 feet. Other ground-water sampling sites were located midway between the subsurface tile drains, and the suction probes were installed at 6-, 30-, and 54-inch depths.

Water inflow to all the ponds was measured by either rotameters or propeller-type flow meters. The surface effluent of the large ponds was measured by Parshall flumes and the subsurface effluent was measured with a bucket and stop watch.

Surface and subsurface effluent samples were obtained by grab and composite methods of sampling from the effluents.

The grass pond studies covered two separate operational periods. In the west plot in 1971, grass was not planted and copper sulfate was applied to prevent weed and algal growth. Both the east and the west ponds were operated at an average depth of 6 inches. In March of 1972, the ponds were dried for releveling prior to the beginning of the second period in July 1972. The west pond was operated as a grass pond with a 3-inch water depth while the east pond was continued at a 6-inch depth.

The operation of the microplots of the grass varieties will be discussed in the results sections.

Analytical Techniques

A well-equipped laboratory was maintained at the Center where a variety of soil and water analyses were performed. Table 6 lists the constituents, frequency, and method of analysis for samples collected during the study. The sampling schedule and frequency of

analysis were based on the variability of the individual parameters, the laboratory capabilities, and the apparent significance of the information.

Table 6. Schedule for chemical analyses

Constituent	Frequency	Method
Nitrate	3-5 times/week	Modified Brucine
Nitrite	3-5 times/week	Diazotization
Ammonia	Once/week	Kjeldahl-distillation
Organic nitrogen	Once/week	Kjeldahl
Orthophosphate	Once/week	Stannous chloride
pH	Once/week	pH meter
Alkalinity	Once/week	Titration-pH meter
Dissolved oxygen	Occasionally	Winkler-Azide modification
Specific conductance	Occasionally	Wheatstone Bridge

Chemical Analyses

The analyses, with some modifications, followed procedures listed in "Standard Methods for Examination of Water and Wastewater," 13th edition (American Public Health Association, 1971). The procedure used for suspended solids in the water followed the procedures of Standard Methods but used Whatman GFA glass filter discs.

Carbon, nitrogen, and phosphorus concentrations in the sludge and soil of the ponds were analyzed at intervals of 2 to 4 months. The methods of analysis of the phosphorus and total nitrogen were according to procedures in "Methods of Analysis for Soils, Plants, and Waters" (Chapman and Pratt, 1961). The procedures used were: (1) soil extraction of total phosphorus with perchloric acid as developed by Sheldon and Harper (1941); (2) analysis of total phosphorus by sodium carbonate fusion after Truog and Meyer (1929); (3) total nitrogen was by Kjeldahl method as described by H. D. Chapman and P. F. Pratt (1961).

The carbon analyses of the soil and sludge samples were performed by the Department of Water Resources Laboratory at Bryte, California, using the Allison Wet Combustion Method as described in "Methods of Soil Analysis" (1965).

Microscopic examination of water was made at 1- to 4-week intervals to determine algal types and estimate populations.

Materials and Methods

Denitrifying Bacteria Counts

A bacterial study program was devised and developed with the advice of Dr. Robert C. Cooper, University of California, Berkeley. The program consisted of two independent but interrelated studies, the "Availability Study" and the "Surveillance Study."

The availability study was used to test the assumption that algae and grass provide sufficient organic carbon for certain bacteria to carry out the denitrification process. Grass and algae samples were either oven dried, autoclaved, or used as wet samples in the tests. The sample of plant material was weighed to determine its concentration of suspended solids. For the wet samples, duplicate samples were dried and weighed to estimate initial dry weight. Water in which the cultures were growing was obtained from IAWTC influent and contained from 18 to 20 mg/l NO_3-N.

Various amounts of the algae or grass were placed in sterilized 300-ml BOD bottles containing 250 ml of the influent water. The remaining air space was purged with 100 percent nitrogen gas to remove all molecular oxygen. The stoppers were inserted and sealed with a high-vacuum grease to limit the entry of atmospheric oxygen. The test flasks were not seeded with bacteria because Phase I and II studies of denitrification had shown tile drainage contained sufficient bacteria for seeding purposes (Sword, 1971). The cultures were incubated at 30°C in the dark and the rate of denitrification determined by daily measurements of the nitrate and nitrite concentration in each bottle. Bacterial counts on selected test bottles were made by the method described below for surveillance study. For comparison purposes, two additional cultures, one with 80 mg/l methanol as a carbon source and one with no added carbon source, were included in each test.

The bacterial surveillance study was devised using the methods described by Alexander (1965) for the enumeration of denitrifying bacteria in soil. Basically, this method uses a growth medium containing asparagine, with bromthymol blue as an indicator. As denitrifying bacteria grow, an accompanying rise in pH causes the solution to change from green to blue.

The qualitative test for the presence of denitrifiers is based on an alkalinity increase associated with denitrification and the formation of gaseous products. This test was modified by Dr. Cooper for use in obtaining some quantitative estimates of population numbers of denitrifying bacteria. The main modification was patterned after a test to estimate coliform group density described in "Standard Methods for Examination of Water and Wastewater" (American Public

Health Association, 1971) and consisted of a 3-tube, 5-step decimal dilution, fermentation test. The test results are converted from the number of positive findings (a color change) resulting from decimal dilution plantings to a "Most Probable Number" (MPN) of organisms. One-milliliter-capacity shell vials were inverted in the fermentation tubes as gas traps for positive observation of gas production. The tubes were incubated in the dark at 30°C and observed for color change and gas formation every 24 hours for a total of 96 hours.

Samples for bacterial analysis were collected at the soil or sludge-water interface, from the water 10 cm above the interface, and from an integrated sample of the total soil water column, using sterile 10-ml pipets. In the grass ponds, the sample column included approximately 3 cm of soil. Three samples of each set were collected, one near the influent, one near the midpoint, and one near the effluent from each pond in the sample program.

Nutrient Regeneration Studies

In these studies, portions of the effluent from selected ponds were placed in the dark and air bubbled through them. The objective was to determine how much of the original soluble organic nitrogen (SON) would eventually break down to ammonia. A controlled environment (light box) was used for a variety of special studies to determine the rate at which certain nitrogen fractions are recycled for further algal growth. Temperature in the light box was maintained between 20 and 25°C and light was provided by cool white fluorescent lights giving 300 to 350 foot-candles at the culture level. The light box was used to test the biostimulating (growth inducing) nature of the SON before and after degradation. The biostimulatory tests were conducted by adding measured portions of the SON-containing solution to flasks and a small inoculum of algae added. Algal growth was followed by measuring daily changes in the fluorescence of the algal cells. Comparisons were then made of maximum algal growth in solutions containing various amounts of SON, before and after degradation.

Additional studies of SON were performed, under contract, by Stanford University. The methods used in these studies have been described by Parkin and McCarty (1973).

Quality Control

Accuracy of chemical analyses was checked by using split samples and reference samples from the EPA, Analytical Quality Control Laboratory, Cincinnati, Ohio. The reference samples provided periodic checking of nitrate, ammonia, and Kjeldahl nitrogen and

ortho and total phosphorus. See table 7 for the results of typical checks. Nitrogen and phosphorus analyses of soil and sludge were performed at the Center and at Bryte Laboratory for comparison of results.

Table 7. Analyses of nutrient reference samples

		Concentration - mg/l			
	Actual[a]	Average and standard deviation[b]			
Parameter	Concentration	May 72	Aug. 72	Nov. 72	Feb. 73
NO_3-N	0.18	0.21±.02	0.24±.01	0.25±.05	0.30±.04
NO_3-N	1.44	1.44±.02	1.99±.02	1.35±.12	1.40±.0
NH_3-N	0.34	0.33±.04	0.34±.01	0.33±.03	0.34±.07
NH_3-N	1.70	1.68±.04	1.63±.004	1.65±.14	1.60±.3
PO_4-P	0.05	0.06±.01	0.05±.0	0.05±.0	0.05±.0
PO_4-P	0.30	0.36±.07	0.42±.01	0.29±.0	0.30±.06
Kjeldahl-N	0.25	0.31±.02	0.32±.003		0.30±.0
Kjeldahl-N	5.25	5.28±.01	4.90±.70	5.03±.32	5.34±1.05
Total P	0.17	0.25±.03	0.12±.05		0.18±.0
Total P	0.85	0.64±.01	0.58±.12	0.26±.02	0.87±.18

a/ As specified by the Environmental Protection Agency Laboratory
b/ Seven replications

In the majority of the analyses, the differences of individual analyses from the numerical average of the determined concentrations fell within the range of accuracy of the test as reported in Standard Methods. The method of phosphorus analysis was changed between November 1972 and February 1973 from that in Standard Methods, 12th edition, to that in Standard Methods, 13th edition; the accuracy of the analysis was improved.

Physical Analyses

A Honeywell continuous recorder monitored sunlight and the temperature of water from the storage pond. Sunlight data from the Department of Commerce, National Oceanic and Atmospheric Administration, Environmental Data Service station at Fresno were compared with the data produced at the Center, and the Fresno values were higher by an average factor of 1.22. The values from the Center are used in this report. A weather station was located onsite and air temperature, rainfall, evaporation, and total wind miles were read and recorded daily. Maximum-minimum thermometers (recorded daily) were located in various algal and grass ponds.

ALGAE-BACTERIA SYMBIOTIC PROCESS

The primary objectives of this study were to determine: (1) the efficiency of nitrogen removal, (2) the manner in which nitrogen was being removed, and (3) the environmental factors responsible for controlling the removal rate. A final objective was to predict the rate of nitrogen removal under various environmental conditions. In the following discussion the algae-bacteria symbiotic process will be referred to as the algal system.

Nitrogen Removal Efficiency

There are two ways to look at the efficiency of a process. One is a mass balance approach:

$$(\text{mass in}) - (\text{mass out}) = (\text{mass removed})$$

The second is a discharge quality approach which compares the effluent concentration to the influent concentration.

At the Center, the net evaporation averaged 0.35 inch of water per day during the period March through September 1972. It is possible to remove a high percentage of the mass and to end up, because of evaporation, with water of poor quality. The following example comparing two ponds at different detention times will demonstrate this paradox of efficiencies:

Detention time - days	16	8
Evaporation (percent of inflow)	50	25
Effluent nitrogen concentration - mg/l	3.5	2.5
Discharge quality efficiency - %	82.5	87.5
Mass removal efficiency - %	91.2	90.7

In the following discussion, because discharge quality is of concern, determination of nitrogen removal efficiency is based on the concentration of nitrogen found in the influent and effluent. Mass emission rates were also calculated as they are useful in estimating environmental effects.

Figure 8 illustrates the total soluble nitrogen concentrations in the influent to and effluent from four of the 1-foot-depth algal ponds, along with their detention times. All four of these ponds were started with layers of soil on the bottom and are representative of what one can expect from the shallow ponds. There was considerable variation in both influent and effluent concentrations; but on an average, the influent contained about 20 mg/l dissolved nitrogen and

the effluent from the most effective (lowest effluent nitrogen) of all the ponds contained about 3 mg/l from April to November, 5 mg/l in December and January, and 4 mg/l in February and March. Detention times necessary to achieve the maximum removals varied from 9 days in the summer to 18 days in the winter. Longer detention times did not improve nitrogen removal in either period. Similar values for the 3- and 6-foot-depth ponds were about 2 mg/l higher, i.e., with a minimum of about 5 mg/l total dissolved nitrogen in the effluent.

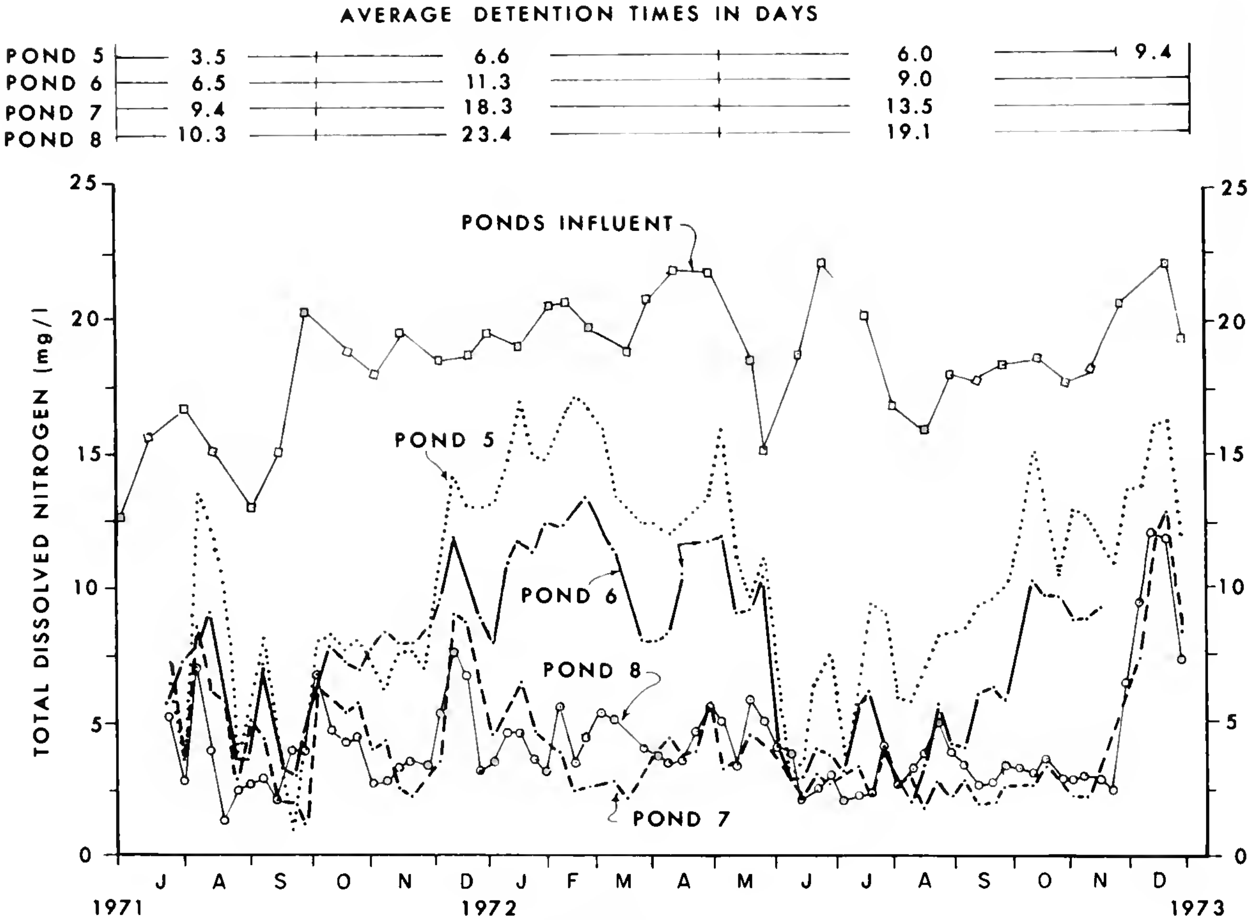


FIGURE 8

TOTAL DISSOLVED NITROGEN IN ONE-FOOT-DEPTH ALGAL PONDS

The data plotted in figure 8 represent the total dissolved nitrogen (which consists of nitrate, nitrite, ammonia, and dissolved organic nitrogen). The influent is about 97 percent nitrate-nitrogen; however, the effluent contains varying amounts of all the nitrogen fractions. Figure 9 illustrates the forms of inorganic nitrogen found in the effluent from a typical algal pond. Normally there was little ammonia present, probably because it was taken up by the algae as soon as it was released. Nitrite-nitrogen was usually found in concentrations averaging about 0.5 mg/l. During the times when the pond was achieving maximum nitrogen removal (March through September 1972), the total inorganic nitrogen was always less than 2 mg/l.

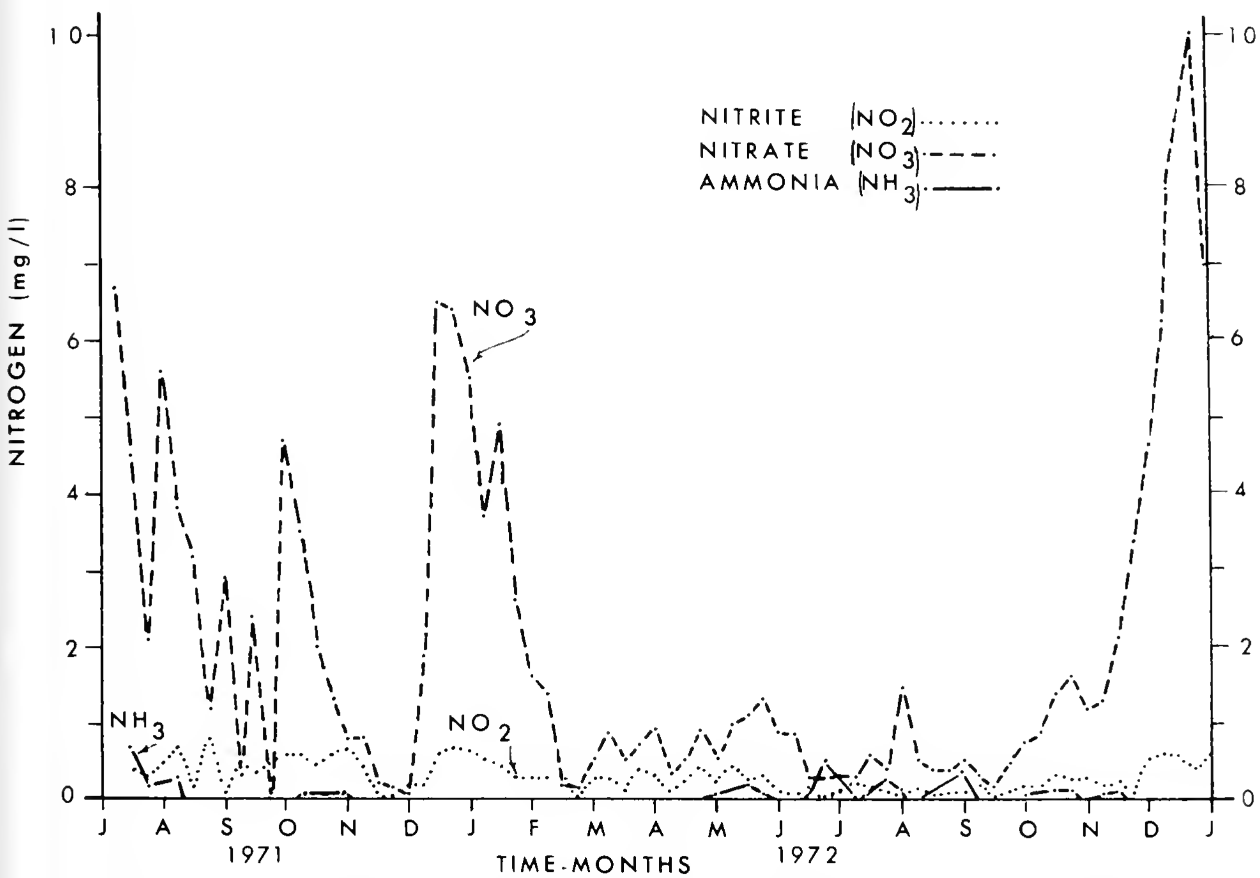


FIGURE 9 FORMS OF INORGANIC NITROGEN IN A TYPICAL ONE-FOOT-DEPTH ALGAL POND EFFLUENT

The concentration of soluble organic nitrogen in the effluent of a 1-foot-deep pond is plotted in figure 10. The soluble organic nitrogen, presumably a decomposition product of algal and bacterial cells plus extracellular products of photosynthesis produced by the algae, was fairly constant for the 17-month period, ranging from 1 to 3 mg/l (average of 2 mg/l). This value was approximately the same for the 3- and 6-foot-depth ponds.

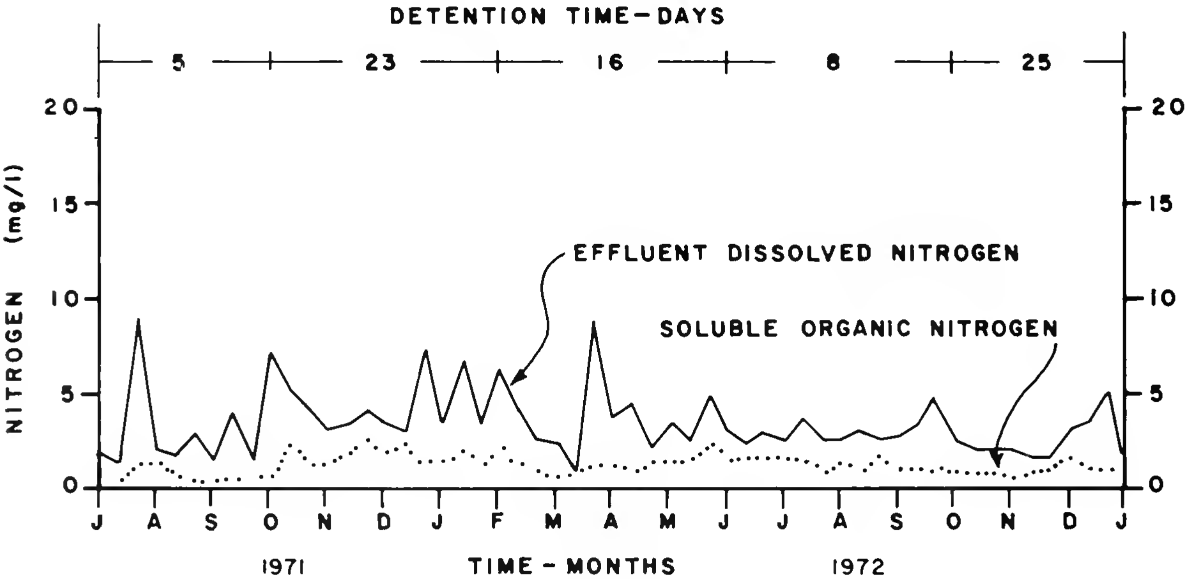


FIGURE 10 EFFLUENT DISSOLVED NITROGEN IN ALGAL PONDS AS AFFECTED BY DETENTION TIME AND SEASON.

The remaining nitrogen component of the effluent was the particulate organic nitrogen contained in the suspended material (algae, bacteria, zooplankton, etc.) leaving the pond. Table 8 contains average values for the particulate material in the effluent, from several of the study ponds. The suspended solids normally were

in the 50 to 100 mg/l range of which about 50 to 60 percent were volatile solids. The concentration of particulate organic nitrogen varied from about 2 to 5 and averaged approximately 3 mg/l.

Table 8. Average suspended solids (S.S.), volatile solids (V.S.), ash, and particulate organic nitrogen of the algae in the pond effluents

Pond	S.S. mg/l	V.S. mg/l	V.S. % of S.S.	Ash mg/l	Ash % of S.S.	Particulate organic nitrogen mg/l	Particulate organic nitrogen % of V.S.
5	62	32	52	30	48	2.8	8.8
6	73	43	59	30	41	3.3	7.7
7	77	46	60	31	40	4.0	8.7
8	112	67	60	45	40	5.1	7.6
11	69	41	60	28	40	3.5	8.5
12	72	41	57	31	43	3.2	7.8
18	64	33	52	31	48	2.5	7.6
21	58	31	53	27	47	2.6	8.4
22	56	30	53	26	47	2.4	8.0
301	47	21	44	29	56	2.0	9.5
302	57	29	52	28	48	3.1	10.7
601	49	29	59	20	41	3.3	11.4
602	55	28	51	27	49	2.2	8.4

The inorganic portion of the total effluent nitrogen was the lowest of the three major components. Based on these kinds of data, it was decided to look more closely at the soluble and particulate organic nitrogen forms and their significance in terms of removal efficiency.

Soluble Organic Nitrogen

The results of the work performed under contract at Stanford University have been described by Parkin and McCarty (1973) and will only be summarized here. The material presented here will be applicable to the grass system as well as the algae.

The soluble organic nitrogen present in the agricultural waste water treated by a symbiotic process consisted of a refractory fraction and a slowly biodegradable fraction. The refractory portion is defined as that fraction which does not decompose biologically within 30 days, and in the case of the pond effluent, represents 50 to 70 percent of the soluble organic nitrogen.

The remaining 30 to 50 percent of the soluble organic nitrogen decomposes within 30 days at rates between 2 and 9 percent per day. The decomposition of the soluble organic nitrogen results in the formation of ammonia which stimulated the growth of algae in laboratory studies. Similar laboratory algal growth potential studies demonstrated that the refractory soluble organic nitrogen did not stimulate algal growth.

The soluble organic nitrogen in the algae pond effluents (and the grass ponds as well) appeared to be similar to the humic material found in soils. From 70 to 85 percent of the material could be removed from the pond effluent by absorption onto activated carbon, and lesser amounts could be removed by chemical oxidation or by coagulation with ferric chloride. None of the removal mechanisms appeared to be economically feasible for preparing waste water for disposal.

The results pertaining to the approximate rate of degradation and the biostimulatory nature of the soluble organic nitrogen fraction were confirmed by additional experiments at the Center.

Particulate Organic Nitrogen

The major contributor to the particulate organic nitrogen fraction of the effluent was the planktonic algal cells. Species present were mostly unicellular flagellate and nonmotile green algae with occasional populations of filamentous green or blue-green algae or diatoms. The predominate flagellated genera were *Carteria*, *Dysmorphococcus*, *Heteromastix*, *Cryptomonas*, and *Pandorina*. Nonmotile genera were *Polyedriopsis*, *Palmellococcus*, *Ankistrodesmus*, *Eremosphaera*, *Schroderia*, and *Scenedesmus*. A small filamentous alga was commonly found in the ponds but could not be identified. It resembled either a blue-green or perhaps the green alga *Hormidium*. Although few in number, diatoms such as *Navicula* and *Nitzschia* were commonly present in the pond effluent. *Polyedriopsis quadrispina* comprised as much as 90 percent of the algal population in new soil-lined ponds for the first 3 to 6 weeks of operation. The chlorophyll content of these algal cells appeared low, giving the water a pale yellow-green color as compared to a lush green color produced by flagellated algae and a dark brownish-green color produced by nonmotile algal populations.

Because the effluent algal cells contained an appreciable amount of organic nitrogen (average of 3 mg/l) an algal harvesting study was designed to determine the type and amount of chemical flocculant necessary to reduce, by settling, the effluent level to 1.0 mg/l particulate nitrogen. Jar test flocculation runs were made from June to November 1972. Three flocculants, ferric sulfate, $Fe(SO_4)_3$;

alum, $Al_2(SO_4)_3$; and lime, $Ca(OH)_2$ were tested for their ability to remove suspended solids from minipond effluent. The amount and estimated cost of each flocculant required to reduce the expected effluent solids seasonally to an effluent particulate nitrogen of 1.0 mg/l is summarized in table 9.

Table 9. Flocculant concentrations necessary to reduce particulate nitrogen in an algal pond effluent to 1 mg/l and the cost* to treat 1 million gallons of effluent

Season	Ferric Sulfate		Alum		Lime	
	mg/l	$/Mgal	mg/l	$/Mgal	mg/l	$/Mgal
Fall	40	6.75	45	20.30	64	5.08
Winter	48	8.16	55	24.57	77	6.14
Spring	43	7.32	49	22.03	69	5.51
Summer	53	9.00	61	27.11	85	6.78

* 1973 prices for the chemicals alone

Summary - Nitrogen Removal Efficiency

Nitrogen removal efficiency is a complex subject. In terms of inorganic nitrogen forms only, the algal ponds can be used to reduce 20 mg/l influent nitrogen to 1 to 3 mg/l depending on the season. However, on the average, about 2 mg/l soluble organic nitrogen and 1 mg/l particulate organic nitrogen (assuming algal harvesting) are also found in the effluent which gives an effluent containing 4 to 6 mg/l total nitrogen.

If the removal efficiency is considered in terms of biostimulatory nitrogen, then the numbers become a little more vague. The 2 mg/l of soluble organic nitrogen is about one-half refractory material (does not break down within 30 days) which is not immediately stimulatory to algal growth. Foree and Barrow (1970) presented data which indicated that approximately one-half of the nitrogen contained in algal cells would not be regenerated in periods averaging almost 300 days. Based on the above estimates and the season, a typical algal pond effluent (after algal harvesting) would contain 2.5 to 4.5 mg/l of biostimulatory nitrogen (1 to 3 mg/l inorganic nitrogen; 1 mg/l potentially stimulatory, soluble organic nitrogen; and 0.5 mg/l potentially stimulatory particulate organic nitrogen). Even this number is slightly misleading since the organic nitrogen fractions must be converted to ammonia to become stimulatory to algae--a conversion rate which is on the order of a few percent of the original amount per day.

As is evident from the preceding data and discussion, the effectiveness of a treatment system for removing nitrogen is only a part of the story. The various nitrogen forms in the effluent and their effect on algal growth in the receiving waters must also be considered.

Nitrogen Removal Mechanisms

Nitrogen removed from the water by the algal system was quantified by determining the distribution of the various nitrogen forms within the system. That quantity of nitrogen not accounted for in the nitrogen balance was considered to have escaped as nitrogen gas released by the denitrification process. Carbon in the system was also mass balanced because the assimilation of inorganic carbon by algae provided the organic material required for bacteria to denitrify the nitrate-nitrogen.

The mass rate of carbon and nitrogen entering and leaving the ponds was calculated from the flow rate and concentration of carbon and nitrogen in the influent and effluent. Mass rates of nitrogen and carbon removed were determined as the difference between the influent mass loading rate and the mass rate of discharge in the effluent, using the equations:

$$\text{MASS LOADING RATE} = Q_i C_i \tag{1}$$

$$\text{MASS RATE OF DISCHARGE} = Q_e C_e \tag{2}$$

Where,

Q_i is the influent flow rate
Q_e is the effluent flow rate
C_i is the influent concentration
C_e is the effluent concentration

and, $$Q_e = Q_i - E \tag{3}$$

Where, E is the net evaporation flow rate.

The effluent contained particulate organic nitrogen and carbon assimilated by the algae and other plankton, dissolved nitrogen, and dissolved inorganic carbon as HCO_3^-, $CO_3^=$, or $CO_2^=$. Particulate organic nitrogen was determined as the difference between total and dissolved Kjeldahl nitrogen, and particulate organic carbon was calculated as 50 percent of the volatile suspended solids concentration.

Algal cells settling to the bottom of the ponds added organic nitrogen and carbon to the sludge layer. The mass of nitrogen or carbon in the sludge was determined by periodic sampling and analysis, and extrapolation of the sample data to the entire pond.

Nitrogen Balance

Nitrogen mass balances were computed for five algal ponds, three with initial soil linings and two without. The data from these ponds are summarized in tables 10 and 11. In table 11 it is apparent that there was some variation in the fate of nitrogen entering an algal pond. Ponds 7, 22, and 12 are most important (to this discussion) because their nitrogen removal efficiencies are about what would be expected from efficiently operated ponds using this process. From these data apparently about 50 percent of entering nitrogen was denitrified, or at least could not otherwise be accounted for. About 15 percent of influent nitrogen left the pond in algal cells and a similar amount was added to the sludge. After operating for a few months, the distinction between soil and no soil became less because the layer of algal sludge, windblown soil particles, precipitates of the added phosphorus, etc., essentially became a soil layer.

The data indicate that nitrogen accumulates in the sludge. In pond 22, for example, the sludge nitrogen increased from about 200 grams in August 1968 to 450 grams in November 1972. Monitoring the effluent during this period of time did not indicate that there were periodic large releases of nitrogen from the sediment or any long-term effects of the soil layer on the fate of influent nitrogen.

Table 10. Grams of nitrogen in various components of five algal ponds during the period November 1, 1971, through November 29, 1972

Component	Pond				
	MP-5 (soil)	MP-7 (soil)	MP-22[b] (soil)	MP-12[b] (no soil)	MP-18 (no soil)
Influent	4,479	1,659	2,469	2,444	2,308
Effluent-					
Dissolved	2,371	343	377	479	1,150
Particulate	496	271	328	348	205
Added to sludge	141	155	450	520	131
Denitrified[a]	1,471	890	1,314	1,097	822

[a] Influent less the nitrogen accounted for in the effluent and added to the sludge.

[b] Ponds continued from the previous studies (Brown, 1971a).

Table 11. Percent of influent nitrogen in various components of five algal ponds during the period November 1, 1971, through November 29, 1972

(The notes in table 10 apply)

Component	Pond					Overall average
	MP-5 (soil)	MP-7 (soil)	MP-22 (soil)	MP-12 (no soil)	MP-18 (no soil)	
Effluent-						
Dissolved	53	21	15	20	50	31.8
Particulate	11	16	13	14	9	12.6
Added to sludge	3	9	18	21	6	11.4
Denitrified	33	54	53	45	36	44.2

The above data on the nitrogen balance indicate that denitrification is apparently the main process by which nitrogen is removed from the influent. Two substudies were conducted to examine this bacterial process.

The first substudy was made to verify that algae do serve as a readily available carbon source for denitrifying bacteria. In previous studies Sword (1971) and Jones (1971) had used methyl alcohol (methanol) as the organic carbon needed by the bacteria to denitrify agricultural drainage. Figure 11 illustrates the fate of nitrogen and the estimated population of denitrifying bacteria in an unseeded culture containing an initial 20 mg/l of NO_3-N and 80 mg/l methanol. By the end of 7.5 days the nitrate and nitrite concentration had dropped to zero, but there was little apparent change in the estimated numbers of denitrifying bacteria. As also illustrated in figure 11, a companion culture which did not contain methanol showed no decrease in nitrate concentration after 8 days; the estimated number of denitrifying bacteria was about the same as when methanol had been added ($\approx 1 \times 10^4$ cells/ml).

The lack of variation in numbers of denitrifying bacteria in the cultures with or without methanol indicated the test being used was not providing a reliable estimate of those bacteria performing, or capable of performing, denitrification. This hypothesis was confirmed when the test was used to determine populations of known denitrifiers. After 4 days' incubation at 30°C, positive results were obtained from _Micrococcus denitrificans_ and _Psendomonas stutzeric_ but not with _Bacillus lichenforms_ and _B. cereus_. In fact, after 14 days' incubation, the results from the _Bacillus_ cultures still did not indicate the

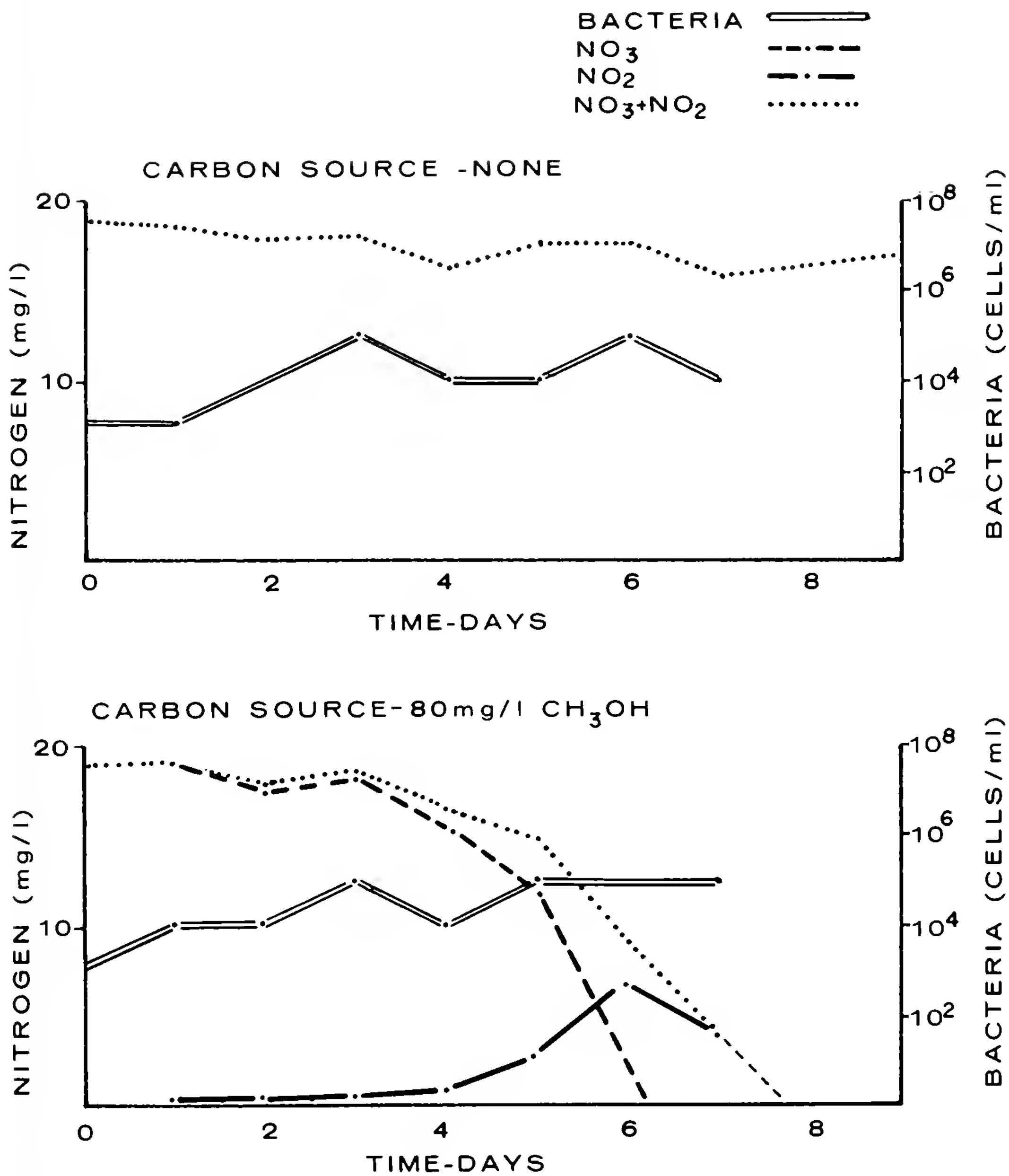


FIGURE 11 NITROGEN REDUCTION AND POPULATION OF DENITRIFYING BACTERIA IN DRAINAGE WATER WITH AND WITHOUT CH_3OH ADDED

presence of denitrifiers. For this reason, the data on bacterial numbers should be considered only as rough estimates.

To test the availability of algae as a carbon source for denitrifying bacteria, aliquots of 50 to 150 mg/l of algae, dry weight basis, were added to bottles containing 20 mg NO_3-N/l (figure 12). Three types of algal products were tested--ovendried, autoclaved, and live. The bottles were stoppered and incubated in the dark at 30°C. There was no apparent difference in the rate of denitrification supported by the various algal products.

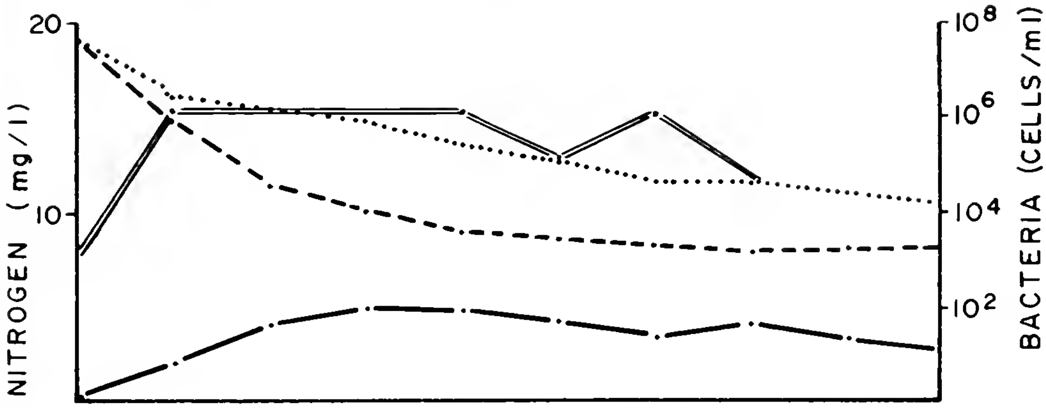
From these data, it appears that 100 to 150 mg/l of algae (dry weight) are approximately equivalent to 80 mg/l methyl alcohol in that, with either carbon source, bacteria can completely denitrify 20 mg/l NO_3-N in 6 to 8 days. The estimated populations of denitrifiers were higher when algae were used as the carbon source as compared to methanol (1×10^7 for algae versus 10^4 for methanol). Why this was so was not determined.

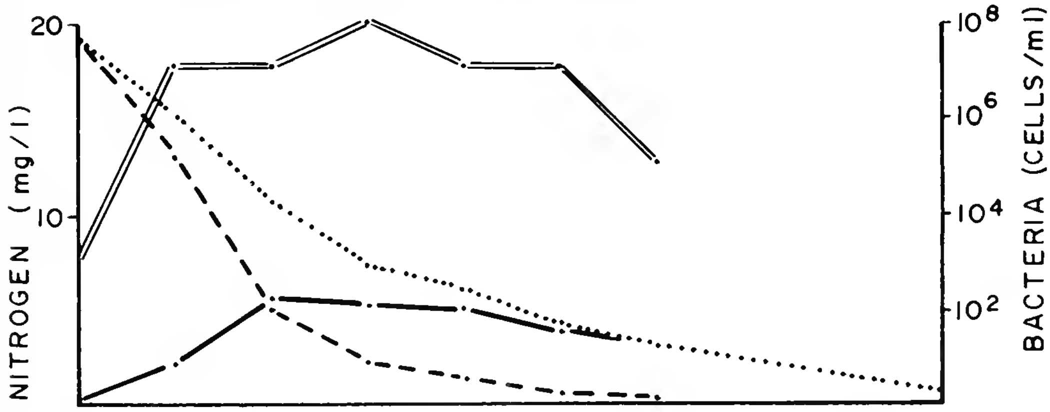
In the second substudy, pond samples were collected from the open water, interface between the sludge and water, and within the sludge itself for analysis of denitrifying bacterial populations. The results indicated that the populations varied from about 1×10^3 cells per milliliter in the water to 1×10^6 in the sludge. Since most of the bacteria were present in the sludge layer, it would seem likely that this was the area of active denitrification. This hypothesis was supported by the fact that there were always measurable concentrations of oxygen present in the open water which would tend to inhibit the denitrification process.

Sludge samples collected from May through December always contained approximately the same number of denitrifying bacteria. Water temperature during this period decreased from about 28°C to 5°C and the rate of denitrification probably decreased during the same period (as evidenced by the increased residence time needed to achieve nitrogen removal). From these observations, it appears that the population of potential denitrifiers remained relatively constant but their actual activity was limited by an environmental condition, probably temperature.

Carbon Balance

In the nitrogen balance studies, the approximate distributions of carbon forms in three of the 1-foot ponds were evaluated. Inorganic and organic carbon forms were determined for the influent, effluent, and sludge layer. Carbon dioxide was determined from alkalinity, pH, and total dissolved solids data using nomographs from Standard Methods (APHA, et al., 1971). Dissolved organic carbon values were not determined. The results of this study are summarized in table 12.





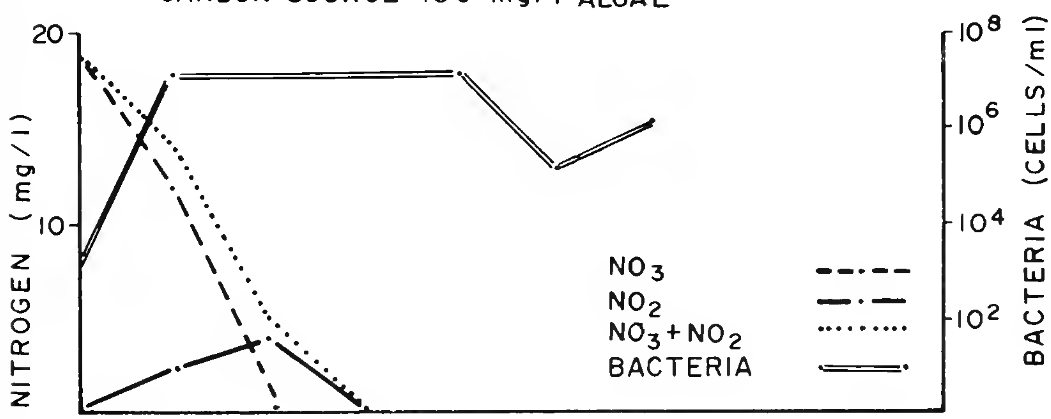


FIGURE 12 NITROGEN REDUCTION AND POPULATION OF DENITRIFYING BACTERIA WITH ALGAE ADDED AS A CARBON SOURCE.

Table 12. Summary of carbon balance in 1-foot-deep ponds
May-November, 1972
(values are grams of carbon)

Source of carbon	MP-5	MP-12	MP-22
Influent	8,500	5,600	5,300
Effluent			
Inorganic	5,000	1,700	2,600
Particulate	1,200	1,400	800
Buildup in sludge			
Organic	1,800	8,000	5,600
Inorganic	3,200	4,900	1,300
Total lost			
(Buildup plus Effluent)	11,200	16,000	10,300
Carbon Balance			
(Influent minus Total Lost)	-2,700	-10,400	-5,000

These data demonstrate a considerable and variable amount of carbon which is not accounted for in the balance. Presumably this carbon entered the pond from the atmosphere. Based on these numbers, the transfer of carbon dioxide from the atmosphere to the pond ranged from about 60 to 330 grams per ft^2 per year. These estimates may be conservative because dissolved organic carbon was not accounted for in the balance. The rate of transfer of CO_2 across the water surface was enhanced by the high pH values, which were often greater than 9.5 in the afternoon.

Inorganic and organic carbon accumulated at different rates in the three ponds studied. As demonstrated by the data plotted in figure 13, the rate of organic carbon accumulation was greatest in the older ponds; probably because they were more active in terms of photosynthetic activity, and their detention time was longer. The rate of accumulation of inorganic carbon was about the same in all three ponds studied; perhaps because the processes involved were mainly physical-chemical influenced by pH, phosphorus concentration, alkalinity, etc.

Phosphorus

Influent phosphorus concentrations averaged 1.15 mg/l after approximately 1 mg/l P (from phosphoric acid) was added. Effluent concentrations averaged less than 0.2 mg/l dissolved PO_4-P (orthophosphorus) and the effluent volatile suspended solids (50 mg/l average) contained about 1.0 percent phosphorus (or about

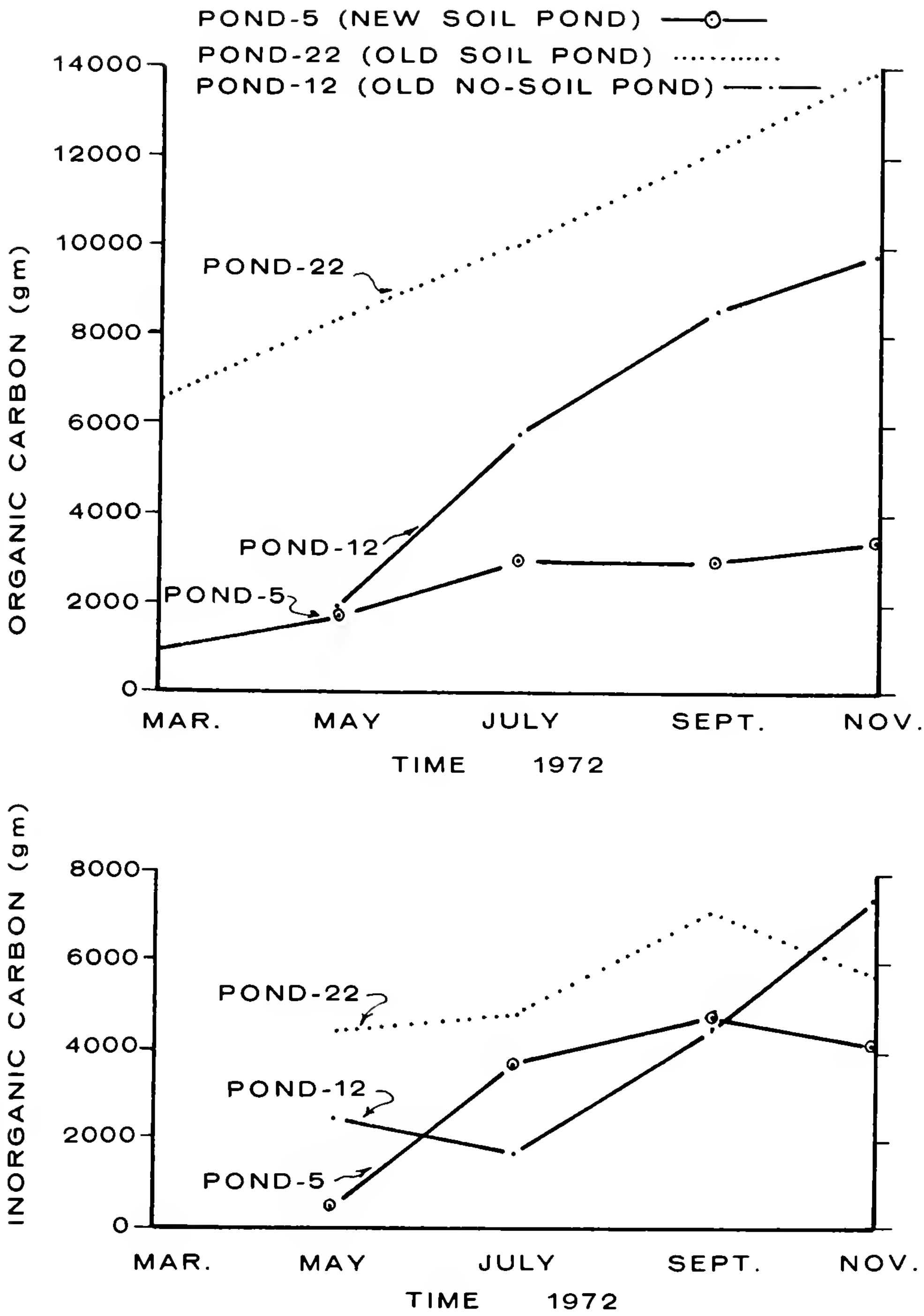


FIGURE 13 CARBON ACCUMULATION IN THE SLUDGE LAYER OF ALGAL PONDS ONE FOOT IN DEPTH

0.4 mg/l organic phosphorus). Analysis of the sludge layer indicated 30 to 70 percent of the influent phosphorus accumulated in the sludge. While a detailed phosphorus balance was not made, the quantity of phosphorus accumulated in the sludge and present in the effluent approximated the mass of influent phosphorus. Most of the residual orthophosphorus in the effluent could be removed either by precipitation in an algae harvesting process or by grass assimilation and soil adsorption in a combined algae-grass pond system.

Factors Affecting Nitrogen Removal

From the preceding discussion it would appear the rate of nitrogen removal would be determined by those factors which would most affect the bacterial and algal growth processes. Possible factors include the rate at which nitrogen is added to the system, culture depth (light availability to the algae), seasonal effects (mainly sunlight and temperature), presence of soil in the pond, and predation by other organisms.

Rate of Nitrogen Addition

The rate at which nitrogen is added to a system is often referred to as loading (amount of material added) per unit of surface area per unit of time. In terms of this report, the units become milligrams of nitrogen per square foot of pond surface per day (mg/ft^2/day).

Nitrogen removal was also considered in terms of milligrams of nitrogen removed per square foot of pond surface per day, and as a rule nitrogen removal increased as surface loading increased. This general trend is illustrated for the 1-foot-depth ponds by the data plotted in figure 14. At surface loadings of 25 to 35 mg/ft^2/day, the rate of removal was 20 to 30 mg/ft^2/day. At loadings of 50 to 70 mg/ft^2/day removal increased to 40 mg/ft^2/day. However, nitrogen removal efficiency (based on the influent and effluent concentrations) decreased from about 80 to 90 percent at the lower loadings to 70 to 80 percent at the higher loadings.

Data from the ponds operated at 3- and 6-foot depths showed the same general trend; i.e., increased mass removal at the higher loading usually accompanied by an increase in the effluent nitrogen concentration.

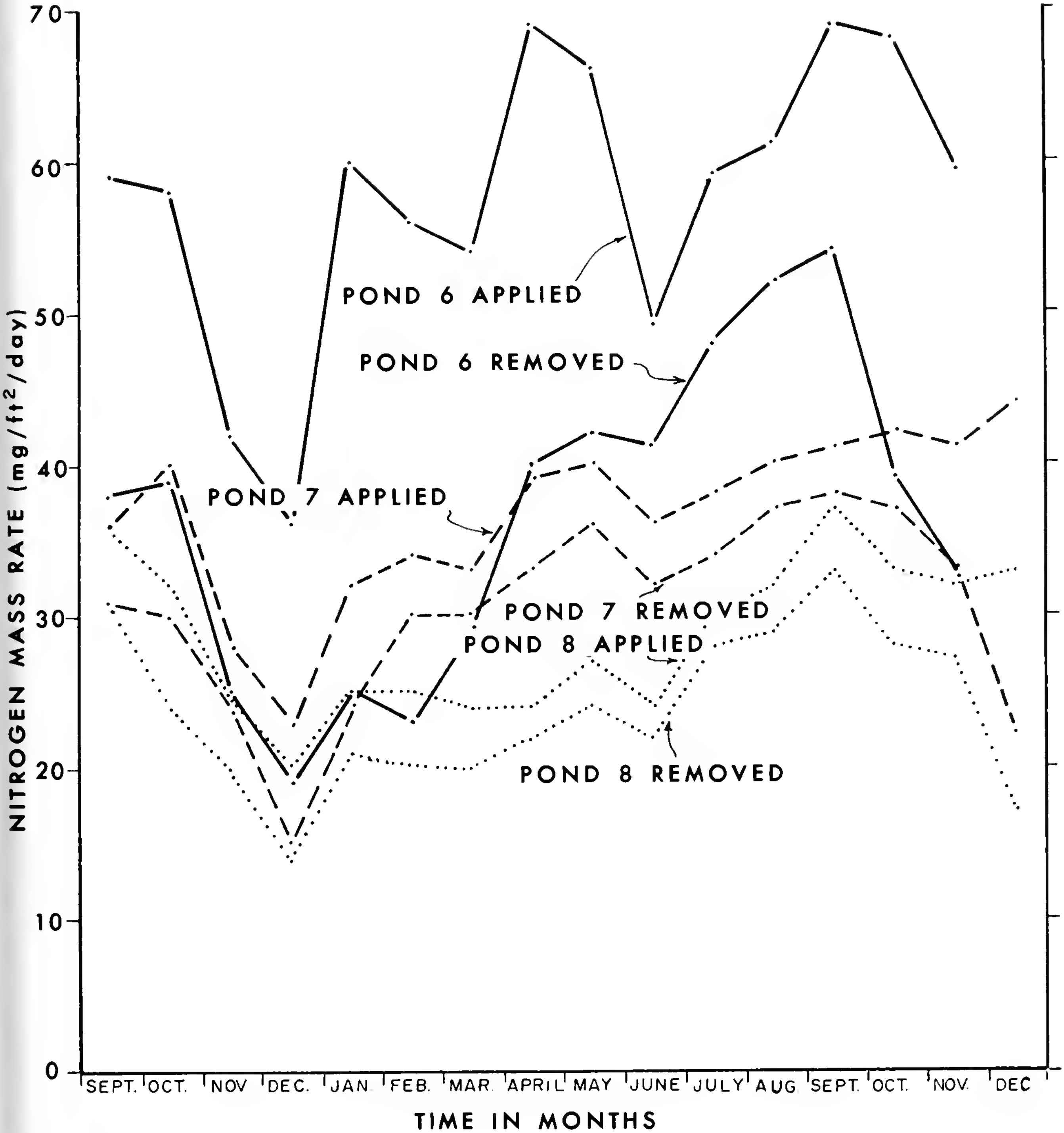


FIGURE 14 NITROGEN REMOVAL AS AFFECTED BY LOADING IN NEW SOIL LINED ONE-FOOT-DEPTH ALGAL PONDS

Algae-Bacteria Symbiotic Process

Culture Depth

Culture depth is important to the symbiotic process in several ways, three of which are: (1) the lack of light penetration into the deeper ponds can limit algal growth, (2) as bacterial action appears to be concentrated in the bottom layer, a large volume of water (and nitrogen) could overload the system; and (3) greater depths and volume of water would be less affected by ambient changes in environmental conditions.

The effect of depth on nitrogen removal was evaluated by concurrently operating 1-, 3-, and 6-foot-depth algal ponds at approximately the same surface loading rates. Two approximate rates were tested, which because of operation problems turned out to be a range of rates; in one case, the loadings were in the 30 to 40 $mg/ft^2/day$ range and in the second, in the 50 to 70 $mg/ft^2/day$ range.

The results of the depth studies for the higher and lower loading rates are illustrated in figure 15. From these graphs it is apparent that when the ponds were operating effectively (the period from March to December 1972) the amount of nitrogen removed per unit surface area per day is apparently independent of culture depth. This observation tends to confirm the earlier conclusion that denitrification is the biological process responsible for removing most of the nitrogen from the system. Bacterial denitrification appears to be more surface and temperature regulated. The algal process can be limited by light availability, and as pond depth increases, light penetration to the lower areas is reduced by self-shading due to surface algal cells.

Even though nitrogen removal per unit surface area was apparently independent of pond depth, the effluent concentration was not. The effluents from the 3- and 6-foot-depth ponds were consistently 2 to 4 mgN/l higher in nitrogen than from the 1-foot-depth ponds.

Climatological Effects

The main climatological factors that affect nitrogen removal are sunlight and temperature. Sunlight directly affects the rate of photosynthesis in plants and temperature has an important role in regulating the metabolic rates of all organisms. Other climatological factors such as rainfall, wind, and evaporation probably are of only indirect importance to the nitrogen removal process. For example, they influence the net outflow from ponds and therefore the degree to which any remaining nitrogen is concentrated in the pond effluent.

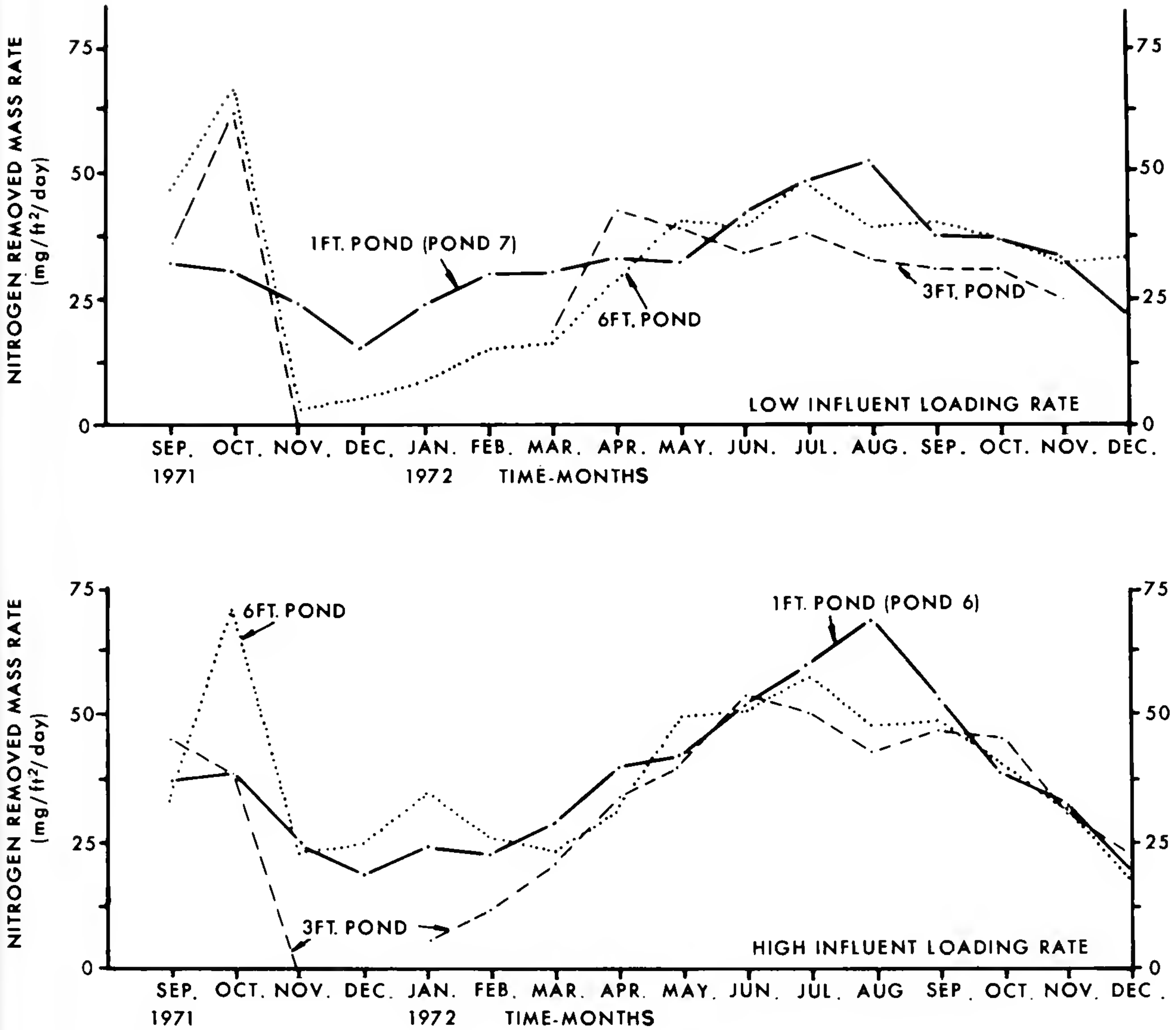


FIGURE 15 THE EFFECT OF DEPTH ON NITROGEN REMOVAL IN THE ALGAL PONDS

The climatological data for the Center are summarized in table 13. The data represent average values for 1-month intervals for the period July 1971 through November 1972 and were used in subsequent regression analyses of nitrogen removal as affected by temperature.

Table 13. Climatological data at the Center
July 1971 to November 1972

Parameter	Minimum	Mean	Maximum	Standard deviation	Yearly total 1972
Air temperature (°C)	5.0	16.7	28.0	7.3	--
Precipitation (inches/day)	0.000	0.018	0.110	0.029	6.6
Evaporation (annual inches)	--	--	--	--	72.6
Net evaporation (inches/day)	0.017	0.181	0.370	0.123	66.0
Sunlight (langleys/day)	106.0	355.0	575.0	165.0	--

The average monthly sunlight and temperature, along with the nitrogen removal from a typical 1-foot-deep algal pond, are plotted in figure 16. As expected, there is a positive correlation between either light or temperature and nitrogen removal. A correlation analysis was run with selected data from the 1-foot ponds (using only those ponds producing an effluent containing 4 mg/l or less total nitrogen). The correlation coefficients between air temperature and total nitrogen removed and again between sunlight and total nitrogen removed were 0.656 and 0.527 respectively. Coefficients of this magnitude indicate a significant correlation but with a large amount of unexplained variation in the data.

Because air temperature and sunlight were not random independent variables, it was not possible to determine which of the two factors was most effective at regulating nitrogen removal. Based on the information presented earlier showing that denitrification was responsible for most of the nitrogen removal, temperature would appear to be the most influential. Figure 17 plots total nitrogen removal versus air temperature for two 1-foot-depth algal ponds (MP 5 and 8). As shown in figure 8 (in the section on nitrogen removal efficiency), the effluent nitrogen values in pond 8 were generally lower than in pond 5. From the plots in figure 17, temperature seems to have a greater effect on the pond producing the highest effluent nitrogen concentration, with the difference between the two curves caused by the higher loading rate in pond 5. The higher loading, which means more removal per unit surface area, is usually accompanied by an increase in effluent nitrogen concentration.

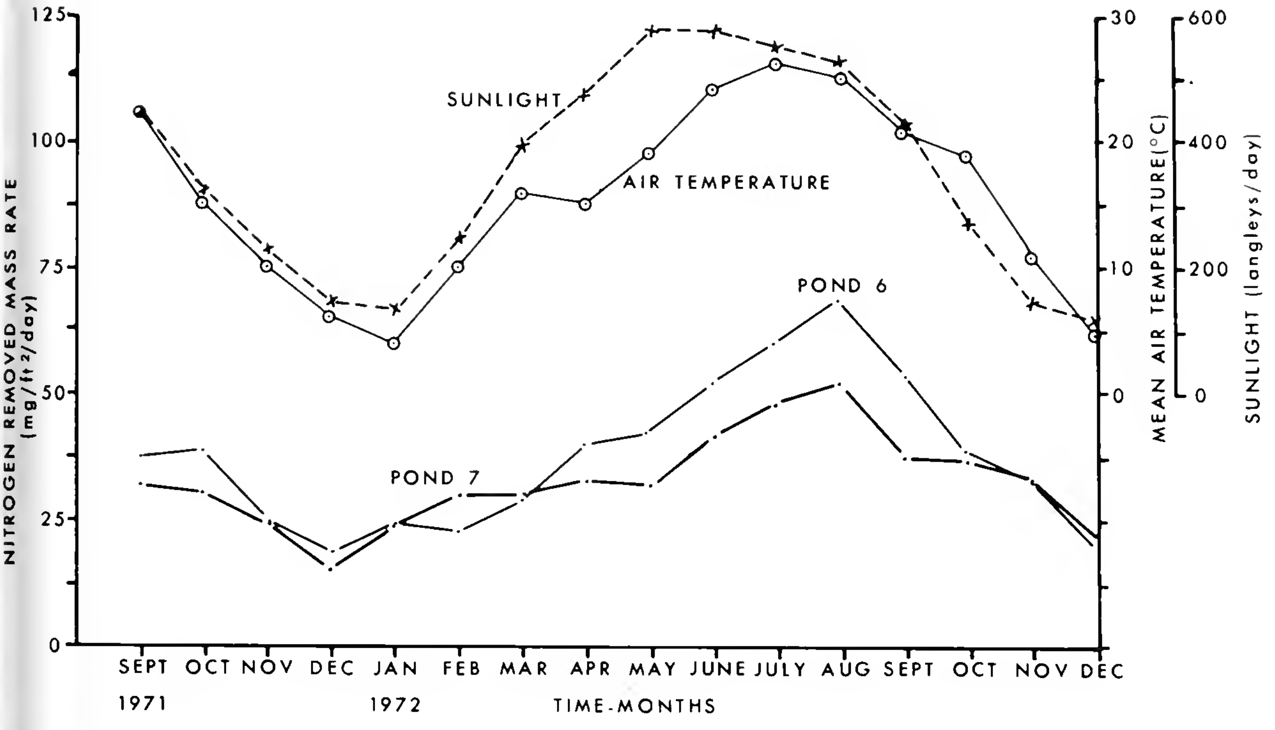


FIGURE 16 COMPARISON OF AMBIENT SUNLIGHT AND AIR TEMPERATURE AT THE TREATMENT CENTER WITH NITROGEN REMOVAL

The data plotted in figure 17 show a definite linear trend; however, there is considerable scatter among the points with a regression coefficient (r) of about 0.6 for either pond. Selected data (a dissolved effluent nitrogen less than 4 mg/l) from all the ponds are plotted in figure 18, with an equation describing the line of least squares fit. Variation among the data with the r value 0.64 means that the equation explained the general relationship of the data, but again there is considerable variation in the data around the computed line.

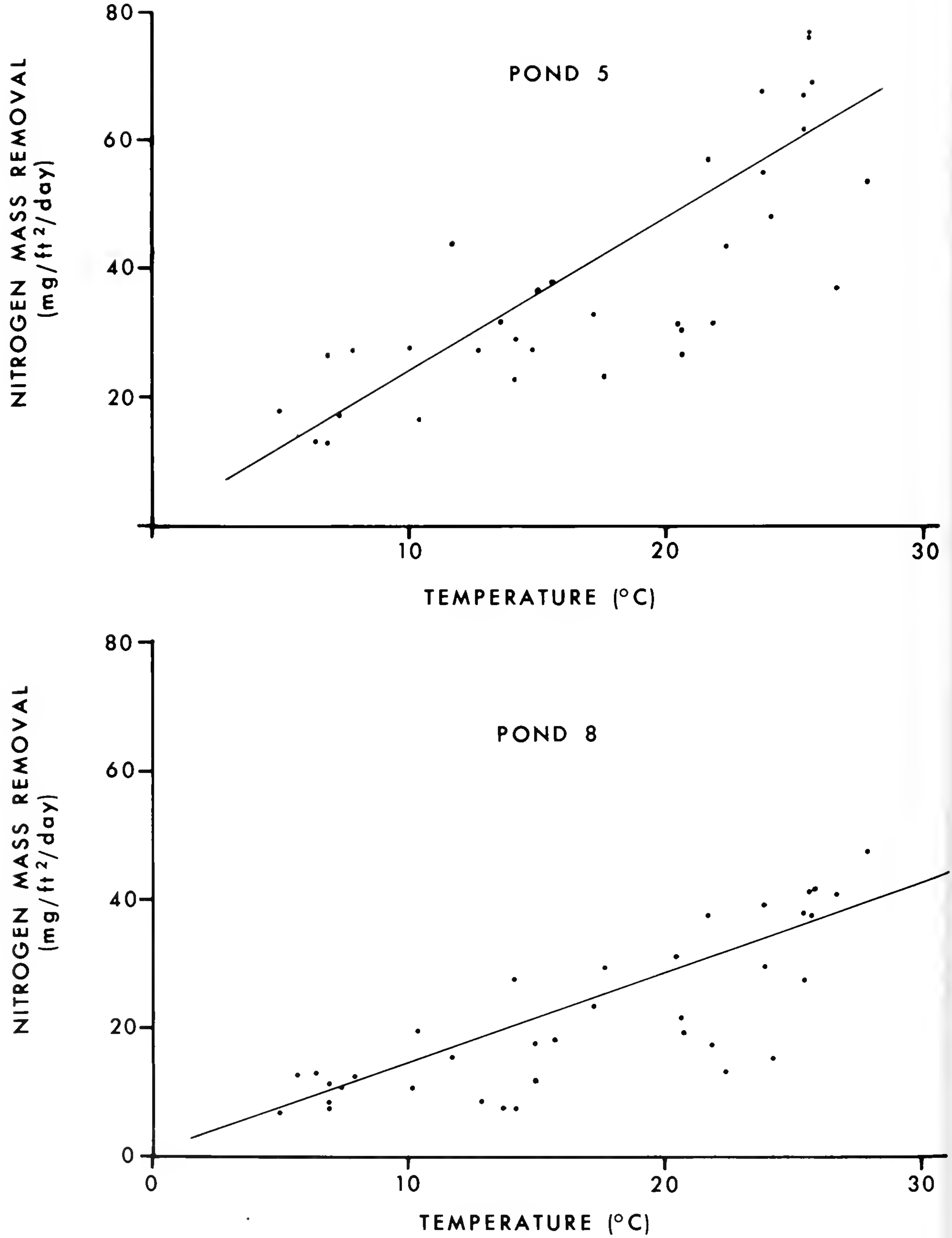


FIGURE 17 NITROGEN REMOVAL VS AIR TEMPERATURE FOR ONE-FOOT-DEPTH ALGAL PONDS.

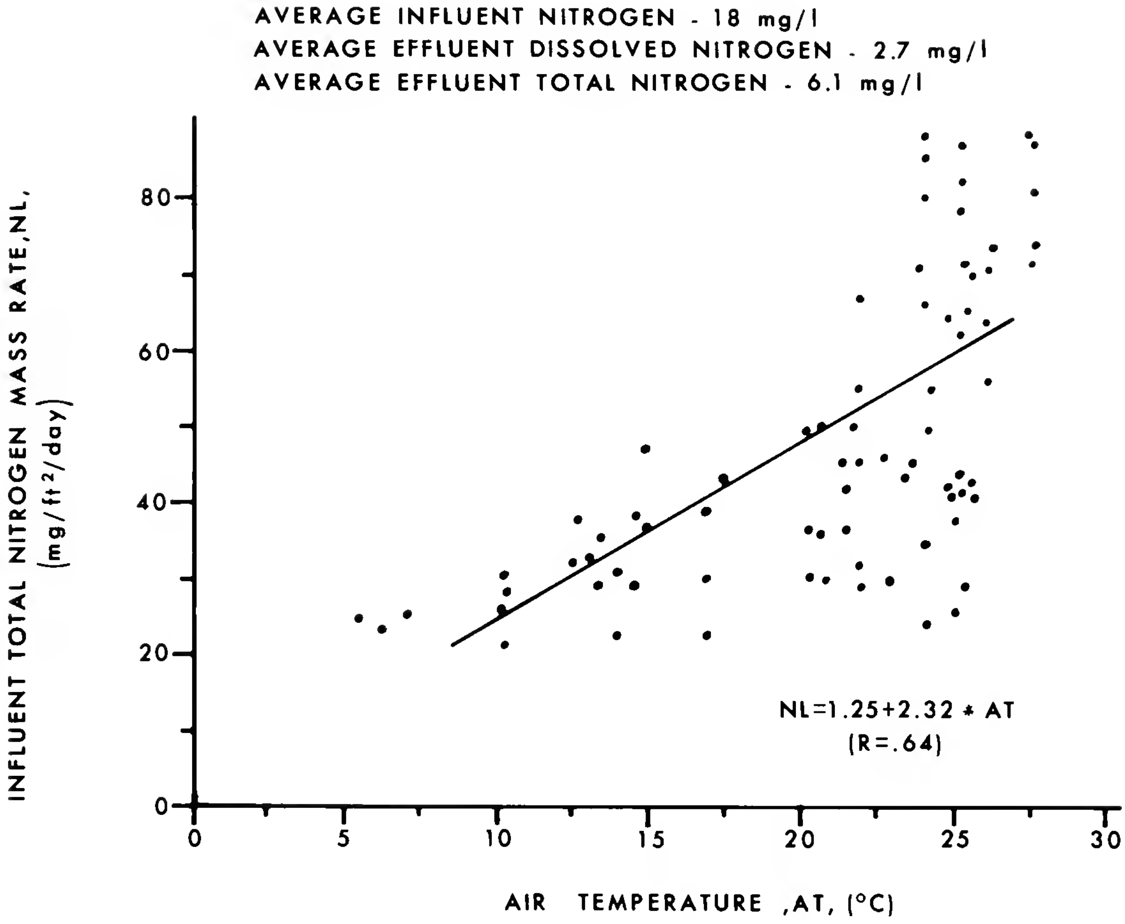


FIGURE 18 NITROGEN LOADING VS AIR TEMPERATURE FOR ALGAL PONDS OF 1-FOOT DEPTH. SELECTED POND DATA CRITERIA: LESS THAN 4mg/l DISSOLVED NITROGEN IN THE EFFLUENT

Another aspect of the seasonal effects on nitrogen removal was that all the 1-foot-depth algal ponds experienced an algal die-off during hot weather in July 1972. Maximum air temperatures ranged

from 40 to 42°C (104 to 108°F) from July 14 to 17. Sunlight averaged 565 langleys per day as compared to more than 600 langleys per day in June. Pond water temperature reached a maximum of 36°C on 3 consecutive days starting July 14. Both motile and nonmotile algal types settled to the bottom of the ponds. The algae also moved below the 1-foot depth in the deep ponds, leaving the upper water clear. By the third day, dead clumps of algae, infested with bacteria, rotifers, and other microscopic decomposers, covered the water surface of the shallow ponds. In about 3 days, decomposition was nearly complete. Figure 19 shows the effect of high maximum pond water temperature and algal die-off on nitrate and ammonia in the effluent. Prior to July 10, or 4 days before the high air temperatures, but only 1 or 2 days before pond water temperatures exceeded 32°C, nitrogen removal decreased. A similar nitrate and ammonia increase was seen in June when pond temperature reached, but did not exceed, 32°C for 3 days, although there was no algal die-off. Ammonia analyses were conducted once each week so the data are less specific as to when NH_3 began to increase. The ammonia increase seemed to follow several days after cessation of nitrate uptake.

An interesting aspect of the shallow ponds failure is a comparison with similar algal die-offs reported by Brown (1971) and Arthur (1971) in the Phase I and II studies. Arthur attributed the cause to an insecticide. However, water and air temperatures were nearly the same as in the symbiotic study die-off. Nitrate uptake also ceased 2 or 3 days prior to the time water temperature reached or exceeded 32° and prior to the possible insecticide dosage. During all three studies, temperature in ponds deeper than 1 foot did not reach 32°C, and there were no long-term adverse effects to the algae or nitrogen removal.

Soil

Two sets of four miniponds, with and without soil lining and operated in pairs at detention times ranging from 10 to 24 days, were used to determine the effect of soil on nitrogen removal. Rates of nitrogen removal, algal population and sludge buildup were compared. Initial algal population in the soil-lined ponds was composed primarily of nonmotile species with *Polyedriopsis quadrispina* dominant for the first 3 to 6 weeks of operation. Flagellated algae increased to a substantial portion of the population after 6 or more months of operation. In the no-soil ponds, flagellated algae were dominant soon after startup with only occasional blooms of nonmotile algae.

Data from two of the shallow algal ponds, operated at equal detention times, but one started with a soil layer and the second

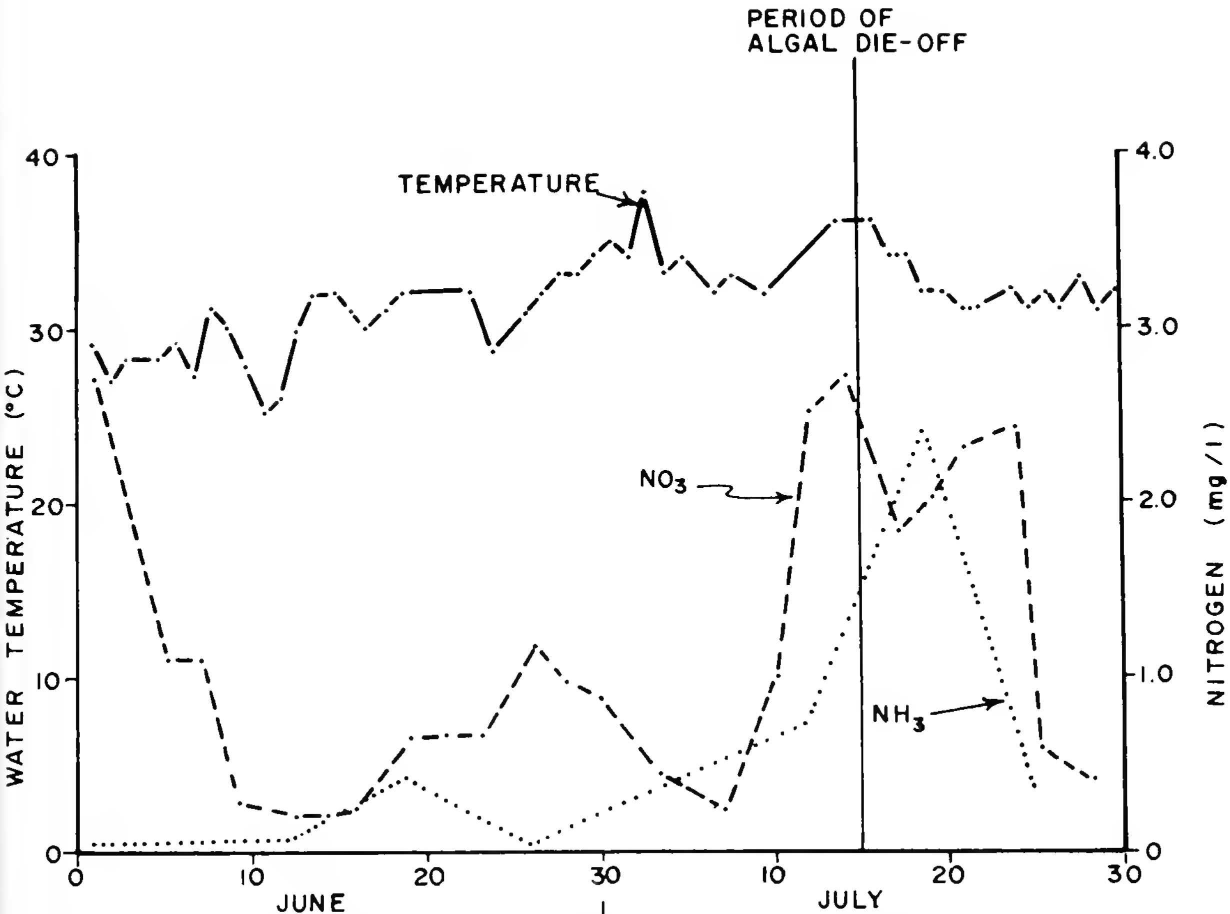


FIGURE 19 EFFECT OF WATER TEMPERATURE AND ALGAL DIE-OFF ON NITRATE AND AMMONIA IN POND EFFLUENT

with no soil, are compared in figure 20. Although there are some inconsistencies, the overall effect of the soil seems to have been to hasten the formation of the anoxic sludge layer on the bottom - the probable site of bacterial denitrification. After 1 year of operation, the two ponds have essentially the same bottom type; therefore, the rate of nitrogen removal becomes the same in both types of ponds.

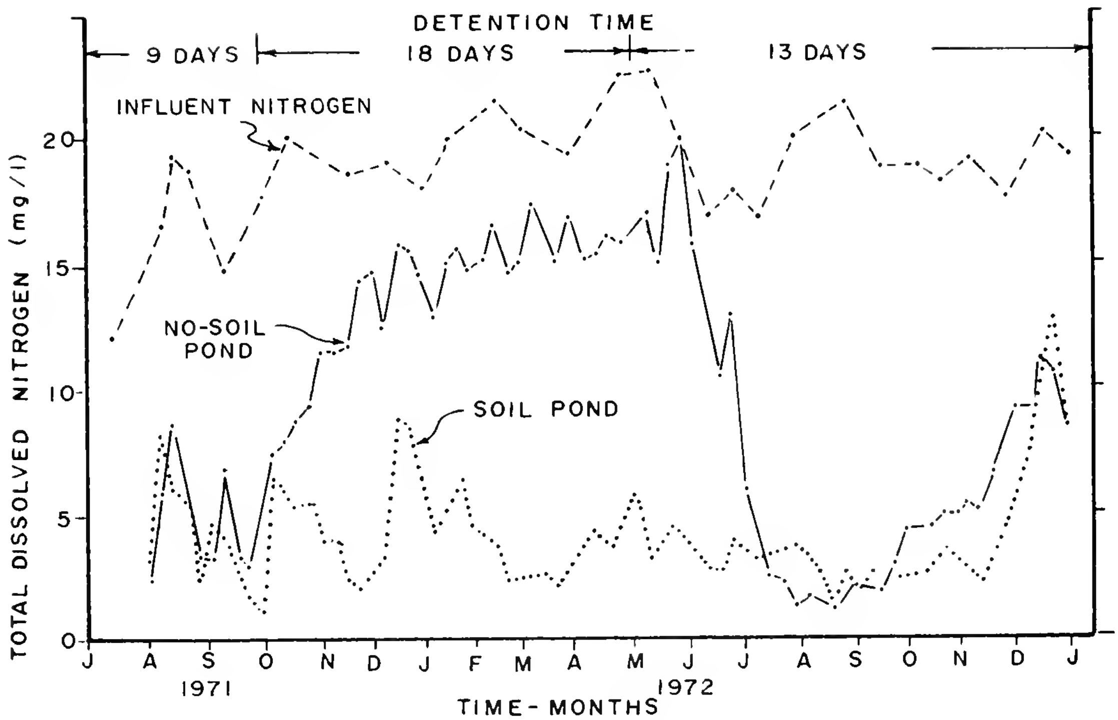


FIGURE 20 EFFLUENT DISSOLVED NITROGEN IN ALGAL PONDS OF EQUAL DETENTION TIME WITH AND WITHOUT SOIL LINING

Predation

Normally predation by zooplankton on algal cells was not a problem in the symbiotic ponds but on one occasion a population of Daphnia apparently almost eliminated the algae in both 3-foot-deep ponds. The problem first occurred in October 1971. Control of the Daphnia in one pond was achieved through the use of a chemical compound called Baytex. The Daphnia persisted in the nonchemically controlled pond until March 1972 when they disappeared.

The Daphnia bloom did show that zooplankton could be a problem in algal ponds, although it never was apparent in the 1-foot-deep ponds. The use of the chemical compound did provide complete and rapid control of the problem once the Daphnia bloom appeared in the deeper units.

GRASS-BACTERIA SYMBIOTIC PROCESS

The grass-bacteria symbiotic process (henceforth referred to as the grass system) was studied using both small (500-ft^2) ponds at the Center and large (3.5- and 3.9-acre) ponds on the Bennett property. In the following discussion, particular emphasis is placed on results from the larger ponds, with data from the smaller units included to confirm or complement. The size of the Bennett ponds makes them more like a prototype pond and in addition the Bennett ponds were not underlain by a water barrier.

The study of the grass system began in the late summer of 1971. Operational problems included poor germination of grass seed because of the late planting, algae and weed control practices, an early frost, and excessive variation in the water depth in the large grass plots. These problems were such that the earliest data collected were not considered representative of a grass system. Necessary maintenance work on the Bennett plot was initiated in March 1972 and the ponds were reseeded with watergrass in May. Flooding commenced in July 1972 with about 3 inches of water in both ponds until July 24, 1972. The depth in the Bennett East pond was then increased to 6 inches and the Bennett West pond left at 3 inches. These depths were maintained throughout the 1972-73 period, except for seed germination in May and June 1973 (data collection was also suspended). The period of July 1972 through October 1973 is emphasized in the data analysis.

As diagrammed in figure 7, both Bennett ponds consisted of several small ponds contained by levees. Water entered the first small pond (called a check), and then flowed in succession through all the checks.

Under this system, as flow rates were constantly being reduced by percolation and evapotranspiration, the flow rates in the first checks were much higher than those in the final checks. Detention times and surface loadings were thus constantly changing. Water might remain in the first check for a few hours but in the exit check for several days. In the grass system, percolation and evapotranspiration were assumed to be evenly distributed throughout the pond. When flow rates were changed either by plan or by natural causes (rainfall, pump problems, etc.) the rate of flow change was observed relatively quickly at the outfall. However, the actual movement of a discrete water mass through the ponds was slower. Because of the large quantities of water leaving the grass system by percolation, the average of the inflow and the outflow was used for the pond flow rate and detention time calculations. As this was not done in the algal system, there is no basis, other than influent loading rate, for correlation between the systems.

Nitrogen Removal Efficiency

As with the algal ponds, a subjective way of looking at removal is to compare the concentration of nitrogen in the pond's influent and effluent. These data for the Bennett ponds are plotted in figure 21.

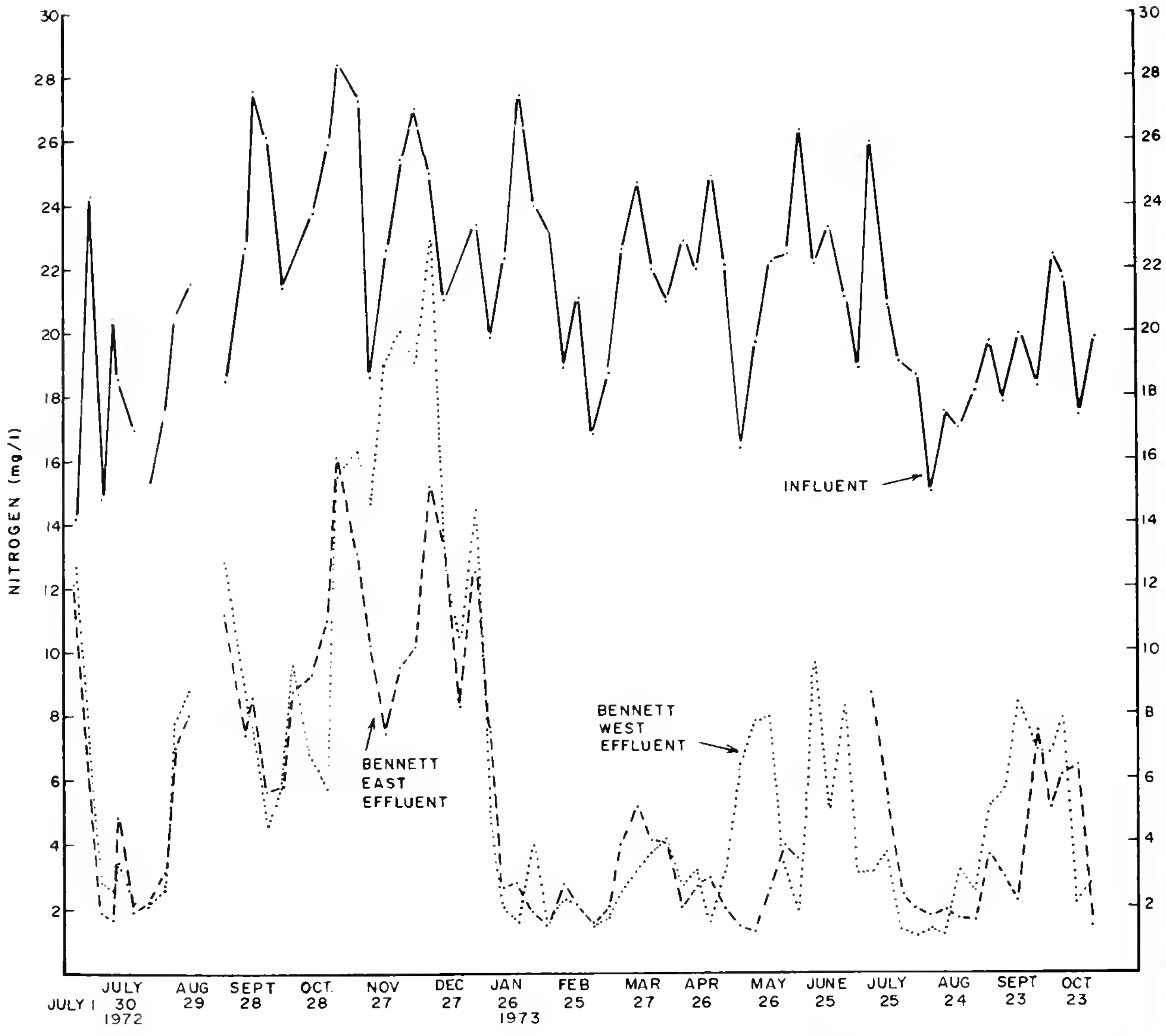


FIGURE 21 BENNETT PONDS - INFLUENT AND EFFLUENT TOTAL NITROGEN CONCENTRATIONS

The changes in effluent nitrogen concentration are influenced by the factors controlling nitrogen removal, principally climate and nitrogen loading. Figure 21 shows that effluent nitrogen concentrations generally were affected by influent nitrogen concentrations. Examples of the interplay of climatic conditions, influent concentration, and flow rate are seen during the period of September 15-28, 1972, when influent nitrogen concentrations increased but effluent nitrogen concentrations declined due to a longer detention time. The high effluent concentrations of December 20, 1972, are in part due to the increased influent nitrogen of December 13, 1972, and to lower temperatures and relatively heavy rainfall from December 16-20, 1972. Rainfall has two opposing effects, dilution and decreased detention times. The decreased detention time influence is more pronounced in the shallower depths. Only for very limited periods of time were effluent total nitrogen concentrations in the range of 2 mg/l a tentative study objective.

Figure 22 shows the influent and effluent monthly average total nitrogen concentrations for two of the small ponds as well as the detention times necessary to reach those effluent concentrations. From February through September 1973, effluent from small pond 4 averaged less than 4 mg/l total nitrogen. The average pond detention times ranged from 5 to 14 days.

The reasons for the relatively high effluent concentrations in 1972 and for pond 3 are explored further in a later section but may have been mainly due to the arbitrary surface loadings used in the study. With greater knowledge of the system, loadings more in line with the system's capabilities would probably be accompanied by a lower and more consistent effluent nitrogen concentration. Also, the influent levels at the Bennett plots were usually higher and more variable than would be expected for a treatment plant operating on a composite valleywide drainage.

As in the algal ponds, the surface effluent nitrogen consisted of soluble and particulate fractions. In addition, the grass plots had subsurface drainage which went to underground collector systems. These items affect nitrogen removal, at least in terms of effective nitrogen removal. In this context, effective nitrogen removal refers to removing (or changing to an innocuous form) that portion of the influent nitrogen which could cause detrimental effects in potential receiving waters.

Surface Effluent-Nitrogen Forms

The nitrogen components of the surface effluent are illustrated in figure 23 for Bennett Pond West during the 1972-73 period. The wide fluctuations in total effluent nitrogen concentration were due

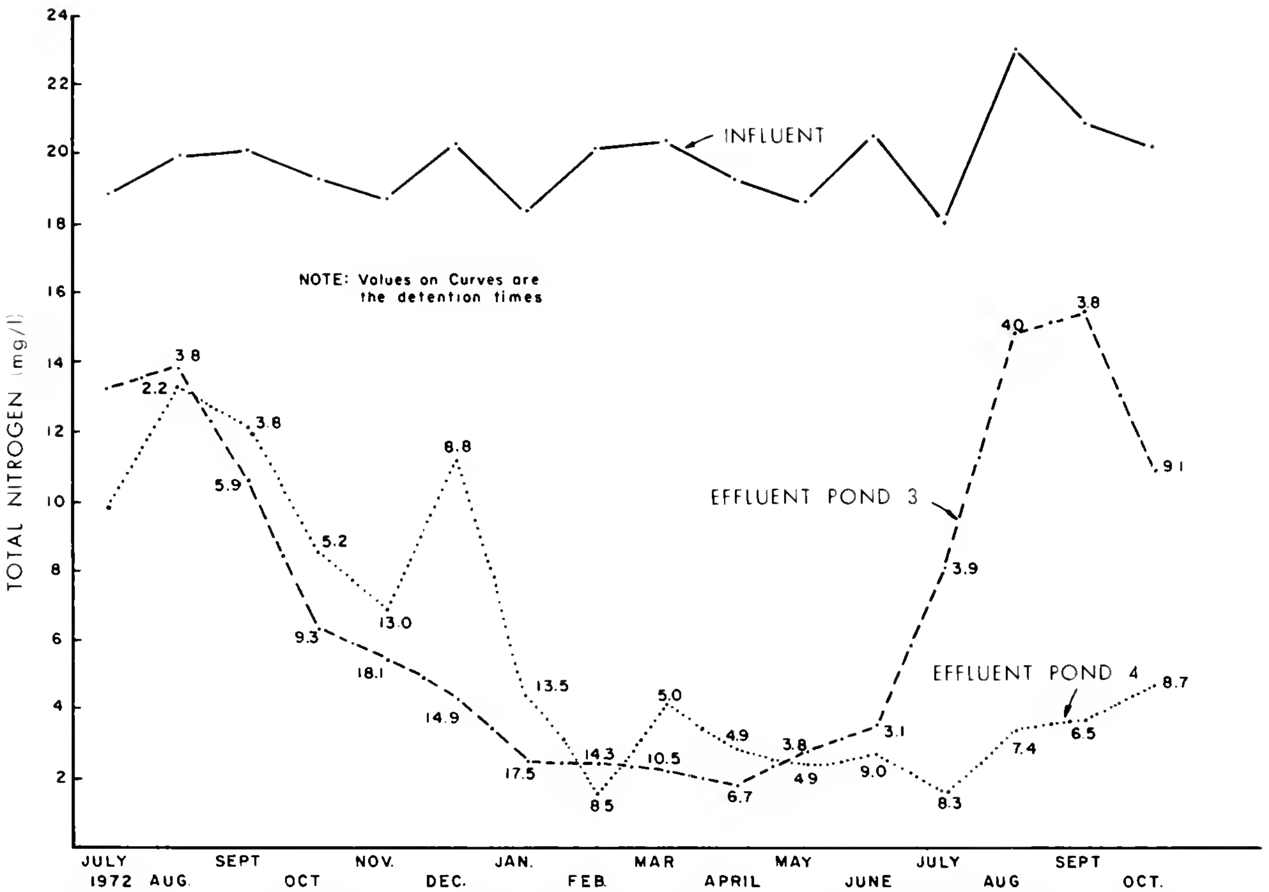


FIGURE 22 INFLUENT AND EFFLUENT TOTAL NITROGEN CONCENTRATIONS AND DETENTION TIMES, SMALL WATERGRASS PONDS 3 AND 4

mainly to variations in nitrate-nitrogen, with the Kjeldahl fraction (ammonia, and soluble and particulate organic nitrogen) remaining relatively constant at about 2 mg/l and nitrite at less than 0.5 mg/l. The Kjeldahl fraction is further broken down in figure 24, demonstrating that the most important component of this fraction was the effluent soluble organic nitrogen. Ammonia levels and the particulate fraction of the organic nitrogen were generally quite low. The low particulate nitrogen fraction was expected since the effluent suspended solids were normally less than 50 mg/l and a few planktonic algae were observed in the grass ponds.

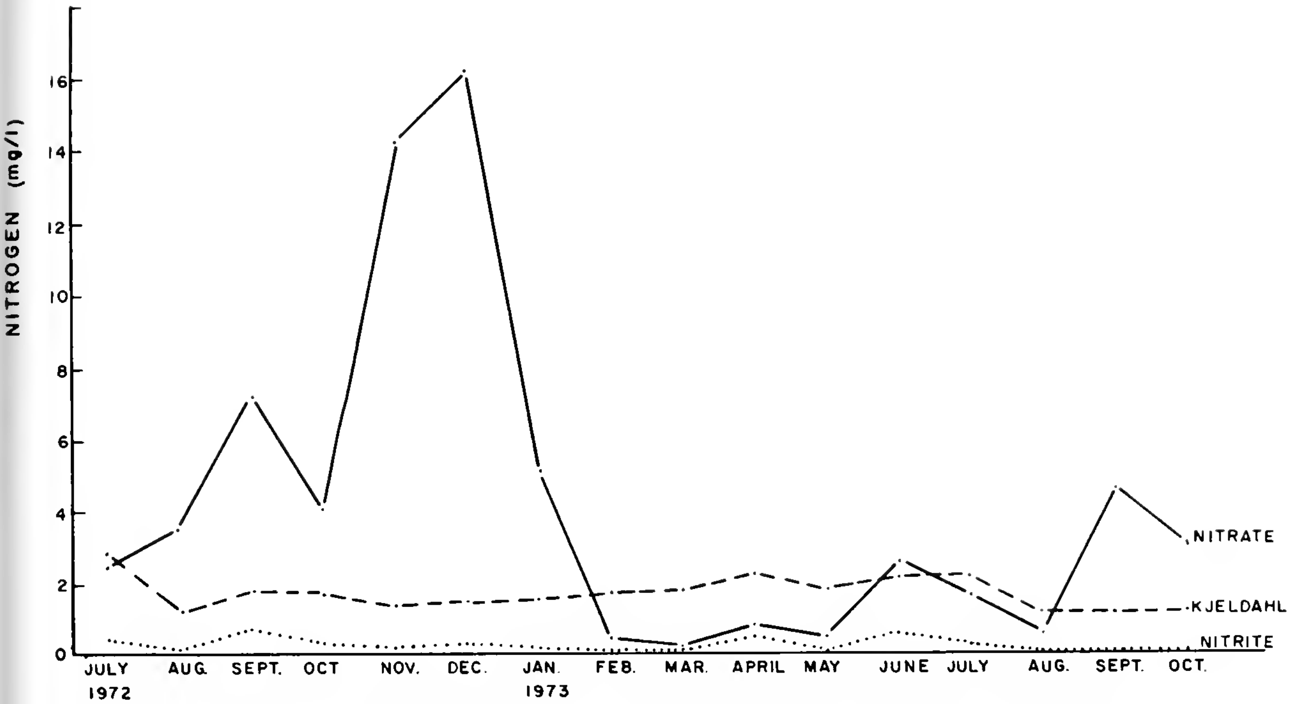


FIGURE 23 BENNETT WEST COMPONENTS OF SURFACE EFFLUENT NITROGEN CONCENTRATION

The data plotted in figure 24 illustrate that the grass process resulted in an approximate doubling of the soluble organic nitrogen. As has been discussed previously, Parkin and McCarty (1973) found that the soluble organic nitrogen was only slowly converted to ammonia. With the relatively short detention times used in this study (3-16 days) it is likely that the influent soluble organic nitrogen passes through the ponds with little change. Any increases resulting from treatment are caused by the decomposition of organisms plus extracellular organic products produced during the in-pond growth of the organism. The discussion of degradability of the soluble organic nitrogen to materials stimulatory to algae growth (see the subsection on Soluble Organic Nitrogen in the discussion of the algal system) are equally applicable to the grass ponds.

The composition of the surface effluent from the four remaining grass plots was essentially the same as that from the pond depicted

in figure 23. The nitrate fraction was variable, while the Kjeldahl fraction was relatively stable at about 2 mg/l. Occasional short-term increases in ammonia and nitrite generally were restricted to the early months of pond operation, after rains, or after a frost. As with the algal ponds, the organic nitrogen prevents complete nitrogen removal.

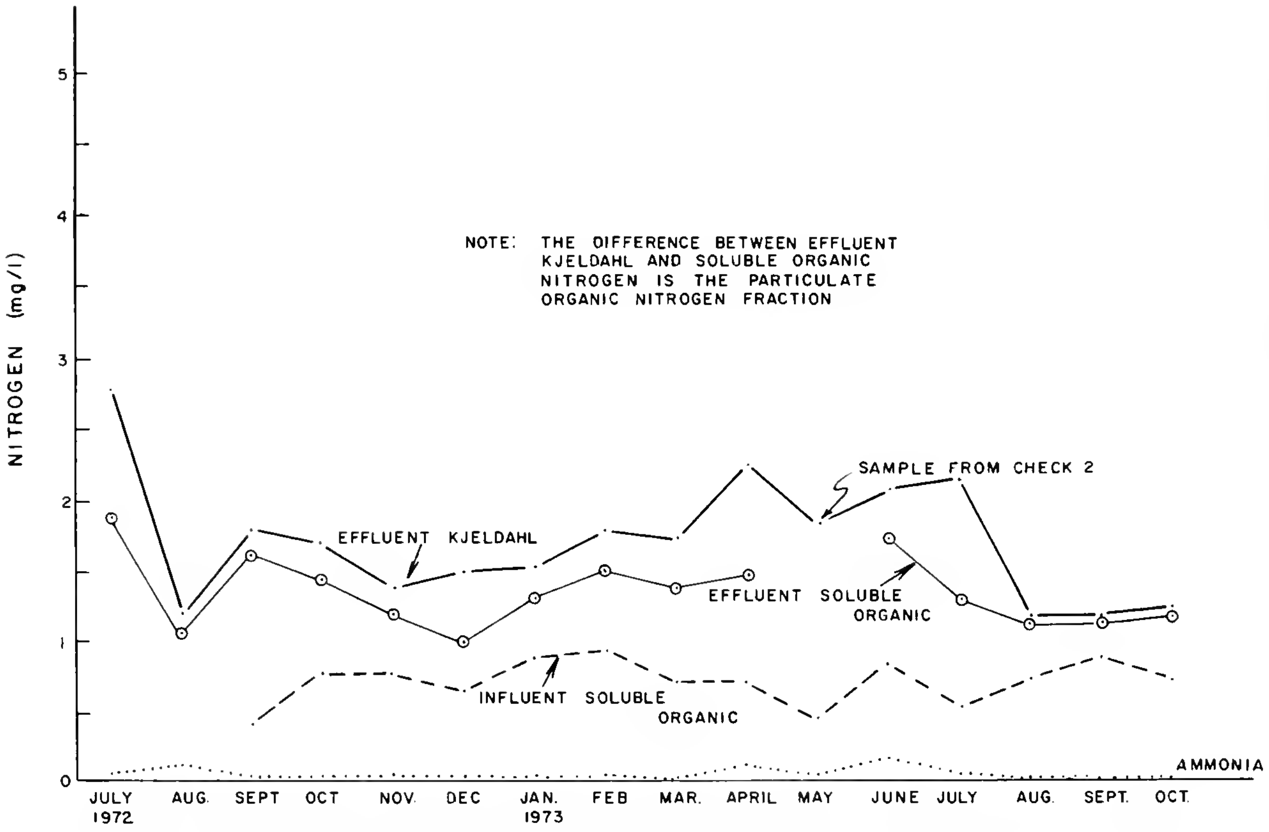


FIGURE 24 BENNETT WEST, INFLUENT SOLUBLE ORGANIC NITROGEN AND COMPONENTS OF EFFLUENT KJELDAHL NITROGEN

Subsurface Drainage

A feature of the grass plots was a system to collect waters percolating from the surface. This feature was not included in the

algal ponds tested (but might be present in an operating algal system, depending on the cost of sealing the pond bottom). The drainage collection system would be installed beneath the ponds to prevent degradation of ground water by the agricultural waste water. In an actual treatment process, the collected drainage would be combined with the surface effluent for ultimate disposal.

Analysis of the drainage flow data from the Bennett Ponds is complicated by the presence of drainage water being drawn in from outside the test plot. The following tabulation of data represents prestudy measurements made 4 to 7 weeks after irrigation in 1971, but before any continuous flooding.

	Drain			
	1	2	3	4
Flow gal/min	1.6	1.6	1.5	2.1
NO_3-N mg/l	1.2	2.1	7.0	3.6
NO_2-N mg/l	.01	.01	.01	.01
Kjeldahl-N mg/l	.19	.50	.59	.43

From these data, substantial background flow containing nitrogen is apparent. There is no historical record of the seasonal variation in drainage quality and quantity.

The monthly average drain flows and nitrogen concentrations (nitrate and Kjeldahl) from the Bennett plot are listed in table 14. Evaluation of the drainage flow data in table 14 is complicated by the fact that most of the water applied on the surface does not reach the drains immediately; it may take several months. It may take an even longer time to leach the native nitrogen from the soil. Looking at the overall averages for flow and nitrate, there was a substantial increase in flow in all drains as compared to background levels and an increase in nitrate concentrations in drains 1 and 2. It is of particular interest to note that by the end of the test period, the nitrate-nitrogen concentrations were uniformly low in all the drains and, except for drain No. 1, were lower than during preflooding. The low nitrogen concentrations in the drains are important to the overall treatment process because drain flows represent a substantial (but variable) portion of the pond effluent.

The drain flow rate will depend on the soil permeability at the treatment site. The dilution capability of the drain flows on the surface effluent will vary between summer and winter. At the Bennett West pond the collected drainage flow was 16 percent of the surface discharge during March through October, and 32 percent during November through February. For Bennett East, the corresponding

Table 14. Average monthly flows from and concentrations of nitrate-nitrogen and Kjeldahl nitrogen in Bennett drains

Month	Drain 1			Drain 2			Drain 3			Drain 4		
and Year	Flow g/m	NO3-N mg/l	K-N** mg/l	Flow g/m	NO3-N mg/l	K-N mg/l	Flow g/m	NO3-N mg/l	K-N mg/l	Flow g/m	NO3-N mg/l	K-N mg/l
7-72		4.2			5.3			6.5			4.4	
8-72	6.7	3.6		10.0	5.4		15.5	4.7		19.0	2.4	
9-72	5.7	3.5		9.3	6.0		12.2	5.0		13.3	1.7	
10-72	4.6	3.3		8.5	6.4		16.8	4.5		11.7	1.8	
11-72	3.7	3.3		5.5	4.6		9.0	4.6		8.6	2.2	
12-72	2.4	3.7		5.0	5.0		7.8	6.3		7.8	3.5	
1-73	3.4	4.0		5.8	6.4		8.4	5.6		8.5	4.6	
2-73	4.8	5.2		6.5	5.4		9.2	5.7		10.2	4.6	
3-73	6.1	5.7		7.1	5.6		10.1	6.6		10.7	3.2	
4-73	5.5	6.4		6.6	4.9		9.5	6.2		10.2	2.6	
5-73	*4.8	5.1		6.0	4.0		9.4	5.2		10.9	1.5	
6-73	5.7	6.1	.35	6.3	2.5	.48	6.9	3.8	.40	*5.8	1.5	.44
7-73	6.2	4.3	.22	6.2	1.9	.29	11.0	3.2	.38	11.5	1.3	.43
8-73	7.9	2.6	.40	8.4	1.5	.45	16.7	2.3	.54	16.1	0.7	.62
9-73	7.3	1.7	.52	9.1	1.1	.57	18.3	1.3	.59	20.4	0.5	.60
10-73	5.3	1.6	.38	7.4	0.9	.40	17.1	1.4	.40	20.6	0.6	.42
Average	5.3	4.0		7.2	4.2		11.8	4.6		12.4	2.3	

* Ponds only partially flooded.
** Kjeldahl nitrogen.

percentages are 53 and 80. Flow balance calculations indicated that additional water percolated past the drains. The total subsurface flow from the ponds in terms of the surface discharge for the same two periods was Bennett West, 31 and 81 percent, and Bennett East, 90 and 200 percent respectively.

Subsurface drainage is not only generally lower than the surface effluent in nitrate-nitrogen, but also contains considerably less nitrite and total Kjeldahl nitrogen. The concentrations of the various nitrogen forms for four of the small ponds are shown in figure 25. One notable aspect of these data is the similarity of effluent quality among the four ponds. In all cases, the average total effluent nitrogen concentration was 1 mg/l or less, and the maximum never exceeded 3 mg/l. In contrast, the surface effluents from the ponds during the same period varied from less than 2 mg/l to more than 15 mg/l.

From the long-term data it appeared that percolating water would contribute very little nitrogen to the final effluent from the grass ponds. This conclusion was substantiated by data from suction probes placed at various locations in the Bennett ponds (figure 7). The 1972-73 nitrate data from the suction probes are summarized in table 15. In general, the nitrate concentrations were uniformly low (less than 2 mg/l) at probes depth from 6 to 42 inches. The only exception was probe site 3 (inlet check for Bennett East), which was consistently higher. An examination of the detailed data for probe site 3 indicated that samples prior to September 20, 1972, had higher average concentrations than the other probes. The surface seals were made more effective and this resulted in average concentrations after this date of 0.93, 1.74, 2.90, 1.62, 1.32, and 4.52 mg/l nitrate-nitrogen, respectively, from the 6-, 18-, 30-, 42-, 54-, and 120-inch probe locations. The samples from 54 and 120 inches may be indicative of a different water source than the upper probes. Table 16 contains some chloride data collected from two of the probes during November 1971. From these data, it appears that the 120-inch suction probe is sampling a different water source than the upper probes. It is also possible that operation of the drain caused different flow lines for water moving through the soil, and that the 54-inch depth was eventually affected by the lower water source.

The major result revealed by the data is the dramatic reduction of nitrogen near the surface in the percolating waters.

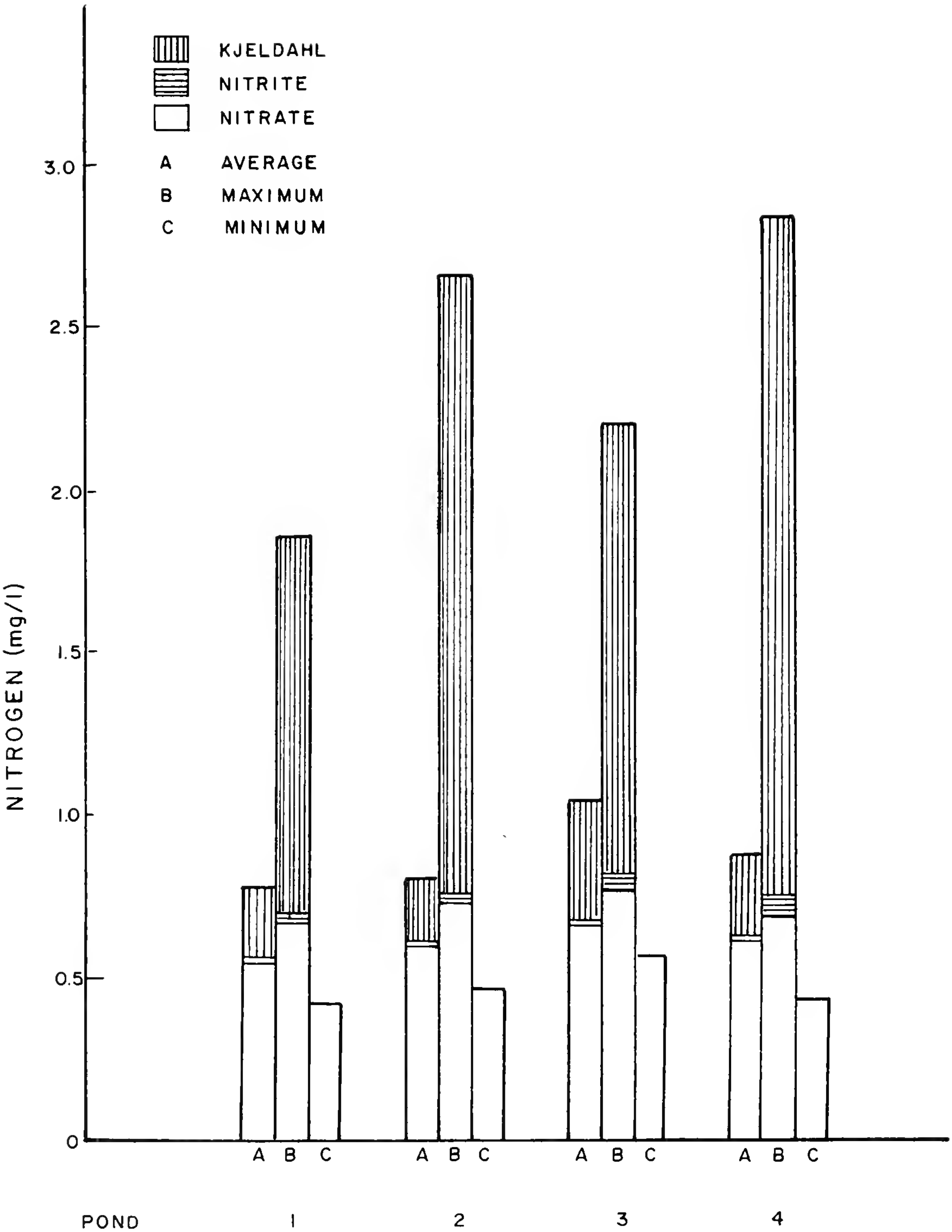


FIG. 25 AVERAGE, MAXIMUM AND MINIMUM NITROGEN CONCENTRATION BY NITROGEN FORMS IN DRAINAGE EFFLUENT OF WATER-GRASS PONDS

Table 15. Average nitrate-nitrogen concentrations in mg/l in water from soil suction probes (1972-1973)

Probe site	Depth (inches)						
	Surface	6	18	30	42	54	120
	mg/l						
1	19.14	.52	1.32	1.99	1.82	4.05	2.31
2	6.55	1.31	1.73	1.27	.91	3.83	1.76
3	19.7	1.63	3.67	5.86	4.50	4.47	4.14
4	4.12	.88	1.73	1.42	1.51	1.33	6.60
5	6.25	1.51		1.18		.82	
6	4.26	1.38		1.31		.33	

Table 16. Chloride results from suction probe sites 1 and 3 November 1971

Date	11/3/71		11/17/71		11/23/71	
Site	1	3	1	3	1	3
	mg/l		mg/l		mg/l	
Surface	585	575	1,284	1,284	553	830
6 inches	851	1,394	1,206	1,383	1,064	1,372
18 inches	1,106	1,394	1,277	1,252	1,184	1,468
30 inches	915	1,511	1,241	1,539	1,096	1,521
42 inches	1,417	1,468	-	1,539	1,298	1,457
54 inches	894	1,149	1,425	1,564	1,234	1,457
120 inches	319	500	808	649	894	-

Summary - Changes in Nitrogen Concentration Through a Grass Pond

The complexities of the algal system are also present in the grass system, with the additional complication of subsurface drainage. Generally the subsurface drainage will contain less than 2 mg/l total nitrogen with about 0.5 mg/l of this Kjeldahl nitrogen. It is assumed this Kjeldahl nitrogen will have about the same degradability rate as the surface effluent Kjeldahl.

The data from the best pond indicate that a surface effluent of less than 6 mg/l total nitrogen was obtained during November and December 1972 and 4 mg/l total nitrogen from January through October 1973. The earlier data in 1972 probably represent startup difficulties. About 2 mg/l of this total is Kjeldahl nitrogen, not all of which is immediately available for use by organisms in the receiving waters.

It is concluded that nitrate plus nitrite nitrogen in the influent water can be reduced to 1 mg/l in the effluent and that Kjeldahl nitrogen will be about 2 mg/l. By blending surface effluent with subsurface effluent, the Kjeldahl nitrogen could be reduced further.

Nitrogen Removal Mass Rates

The preceding paragraphs discussing the grass system demonstrate that the incoming nitrogen concentrations can be reduced. To more exactly compare the ponds and to provide data from which to size the treatment system, the mass rates of nitrogen removal per unit pond surface area have been computed and plotted.

As previously noted, water entering a pond is retained for a period of time prior to discharge. This time lag creates difficulties in the computation of the mass removal rate, but from the analysis of preliminary data (1971-1972) shown in table 17, it was concluded that nitrogen removal could be reliably calculated using influent and effluent data for the same day. In other words, any effects caused by the time lag within the ponds could be ignored.

Table 17. Nitrogen removal from Bennett ponds 1971-72 using current and time delay analyses

Month	Bennett West			Bennett East		
	Detention time days	Nitrogen removal $mg/ft^2/day$		Detention time days	Nitrogen removal $mg/ft^2/day$	
		Current	Delayed		Current	Delayed
August	3.1	55.3				
September	4.3	54.1		4.9	48.1	
October	7.1	19.0	24.1	4.8	23.9	
November	19.3	21.6	19.4	10.0	18.8	24.8
December	10.5	27.4	34.5	17.9	30.6	27.2
January	20.9	19.3	20.5	20.7	26.1	28.6
February	22.1	18.3	*	19.6	24.4	*

*Inflow stopped February 24, 1972; delayed analysis could not be made.

Nitrogen Removal

Figure 26 contains a plot of the mass rate of nitrogen removal achieved by the Bennett ponds. The data for Bennett East were not collected during the period of seed germination. The mass rates of removal were similar in both ponds, even though Bennett East was

maintained at 6 inches water depth, compared to about 3 inches in Bennett West. As might be expected, peak mass removal occurred during May, June, and July when temperature and sunlight were maximum. The data for 1973 are probably more representative of what can be expected from this system, perhaps because the grass crop (planted in May) had not grown sufficiently in 1972 to provide an organic carbon source for the denitrifying bacteria. Support for this conclusion is

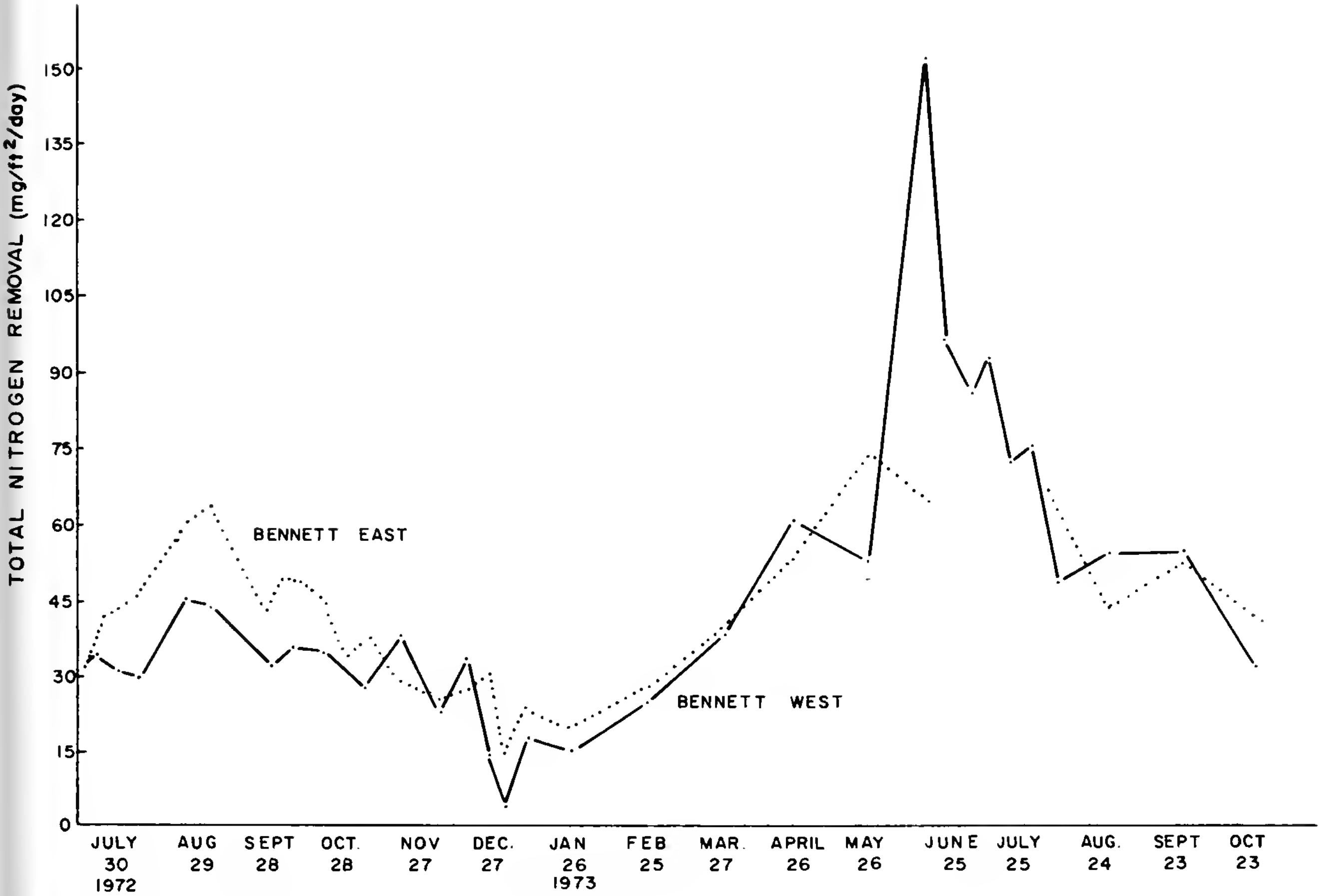


FIGURE 26 BENNETT PONDS, NITROGEN MASS REMOVAL RATES

provided by figure 27 where nitrogen removal data have been plotted for four of the small ponds originally planted with watergrass. In

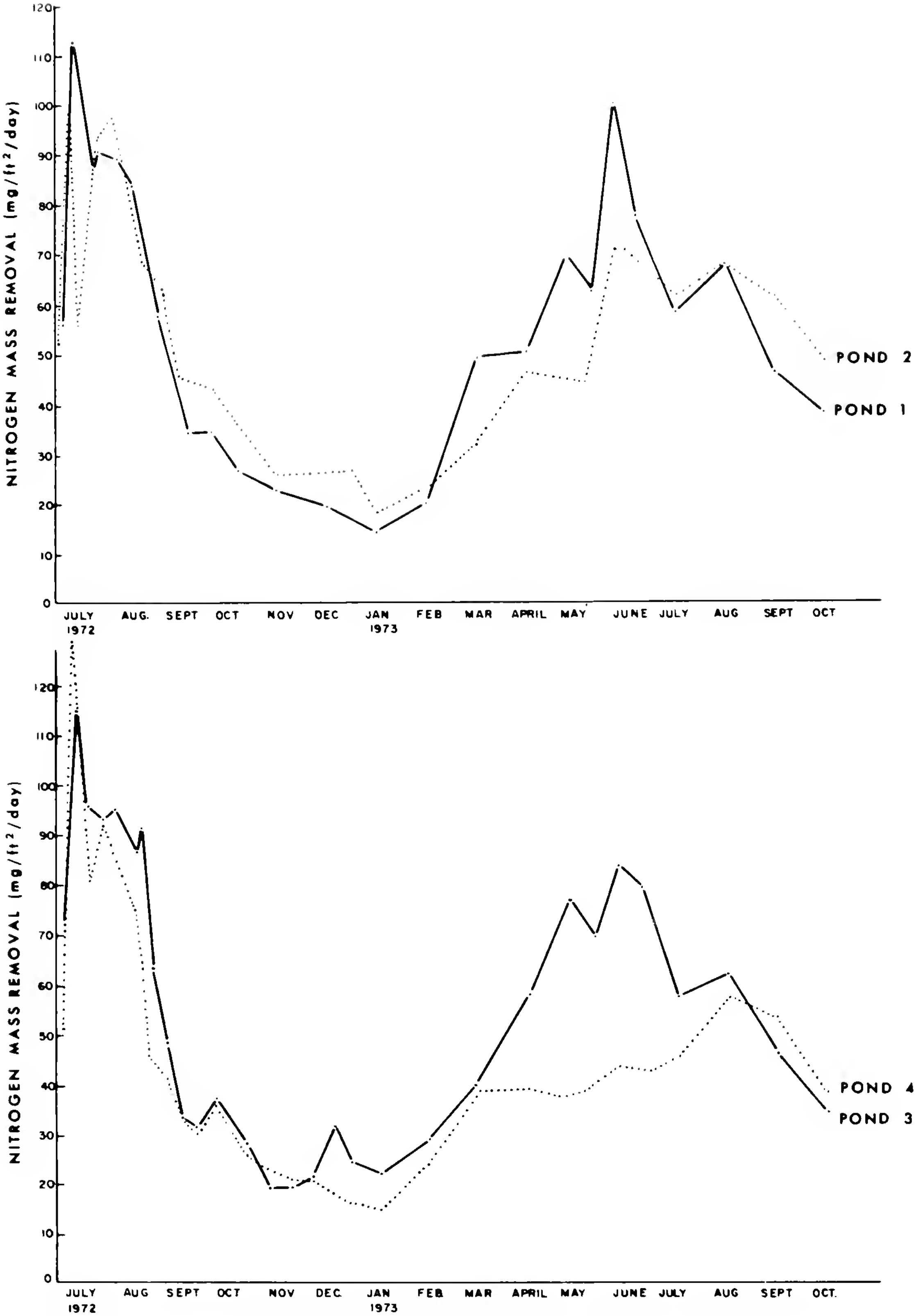


FIGURE 27 NITROGEN REMOVAL FROM WATERGRASS PONDS

all four ponds, the mass rate of nitrogen removal was as high (actually slightly higher) in 1972 as in 1973. Figures 26 and 27 indicate the similarity among mass removal rates at any given time of both sizes of ponds.

The mass rate of nitrogen removal in the Bennett ponds varied from a maximum of about 150 $mg/ft^2/day$ in May-June, to a minimum of 15 to 20 $mg/ft^2/day$ in December-January. In the small ponds, the maximum rate was somewhat lower (approximately 100 $mg/ft^2/day$), perhaps because the Bennett ponds had more subsurface drainage which permitted a greater loss of nitrogen from the ponds. Using data from either size pond, nitrogen mass removal in the grass ponds was significantly higher than the maximum of about 75 $mg/ft^2/day$ reported earlier for the algal ponds.

Comparing the removal rate data with the observed effluent nitrogen concentrations in figure 21, there is, as expected, a general inverse relationship between removal rate and effluent concentration; i.e., higher removal rates mean lower effluent nitrogen concentrations.

Nitrogen Removal Paths

Nitrogen entering the ponds will leave by one of five pathways: plant uptake, denitrification, soil addition, surface outflow, or subsurface outflow. The last two have already been discussed in the section on nitrogen removal efficiency.

Plant Uptake

As the preferred fertilizer for rice is the ammonium form, it was assumed that watergrass would have a similar preference. The data have indicated that nitrate-nitrogen of the order of 0.5 to 1 mg/l reached the drains, so a greater concentration could have reached the root zone where it would be available for plant assimilation along with other available forms of nitrogen. To determine the magnitude of removal by plant assimilation, crop production was estimated by harvesting small areas and determining the amount of nitrogen in samples from these harvests (table 18).

As shown in table 18, production of dry matter and the nitrogen content varied widely. The nitrogen content variation is probably due to varietal difference. In 1972, Bennett West samples contained only watergrass, whereas the Bennett East crop sample contained some sprangletop. For 1973 the Bennett East sample was almost 100 percent sprangletop. The four grass ponds at the Center were wholly watergrass in 1972, but in 1973 were more than 75 percent alkali bulrush. This plant succession will be discussed in more detail in the section on salinity response. Differences due to sampling also contributed to the variation in nitrogen content.

Table 18. Crop production and nitrogen content

Pond	1972 Yield lbs/acre	1972 % N	1972 N lbs/acre	1973 Yield lbs/acre	1973 % N	1973 N lbs/acre
Bennett East	17,733	.857	152	8,600	0.55	47
Bennett West	31,017	.94	291	9,400	.94	88
Grass Pond 1				12,200	1.20	146
2				10,800	1.20	130
3				10,800	1.20	130
4				9,600	1.20	115
Reed canary-grass 5				22,600	1.19	269

The time for assimilation of the nitrogen shown in table 18 is estimated at 100 days for all except the reed canarygrass. For the latter grass, there was probably some assimilation throughout most of the year but with lesser amounts during periods of slow growth and during the frost period. Estimated rates of nitrogen removal by plant assimilation are shown in table 19.

Table 19. Nitrogen removal rates by plant assimilation*

Pond	1972	1973
	mg/ft^2/day	
Bennett East	15.8	4.9
Bennett West	30.3	9.3
Grass pond 1		15.2
2		13.5
3		13.5
4		12.0
Reed canarygrass		7.7

*Assumes even growth rate over 100-day growing season for watergrass, sprangletop, and alkali bulrush and 365 days for reed canarygrass. The estimated range for reed canarygrass is 2 mg/ft^2/day in December and January and up to 12 mg/ft^2/day in the summer.

Denitrification

Denitrification is considered to be the process removing any influent nitrogen which cannot be accounted for in the effluent,

sludge layer, or plant biomass. As was pointed out earlier, denitrifying bacteria require an organic carbon source in order to convert nitrate to nitrogen gas. In the grass symbiotic process, the dying grasses were assumed to provide the carbon source. To demonstrate that grass or an organic carbon source was necessary for the process, one of the small ponds was completely covered and the influent and effluent nitrogen concentrations monitored. These data, plotted in figure 28, demonstrate that in the absence of plant growth, nitrogen reduction did not occur. Figure 29 contains data from the control (covered) pond drain and shows that nitrate concentrations were significantly reduced as the influent water percolated through the soil column. Denitrification was probably accomplished by bacteria using native organic soil carbon.

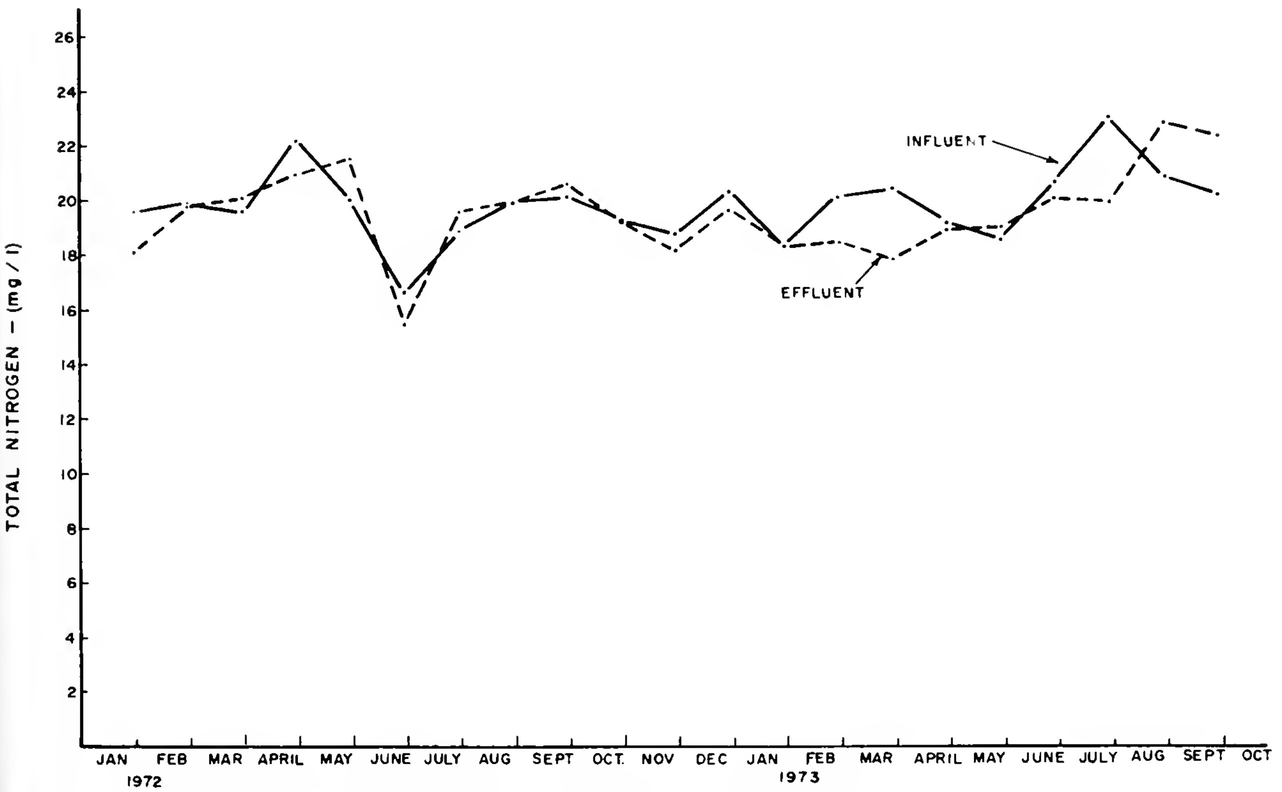


FIGURE 28 SMALL CONTROL POND INFLUENT AND EFFLUENT TOTAL NITROGEN

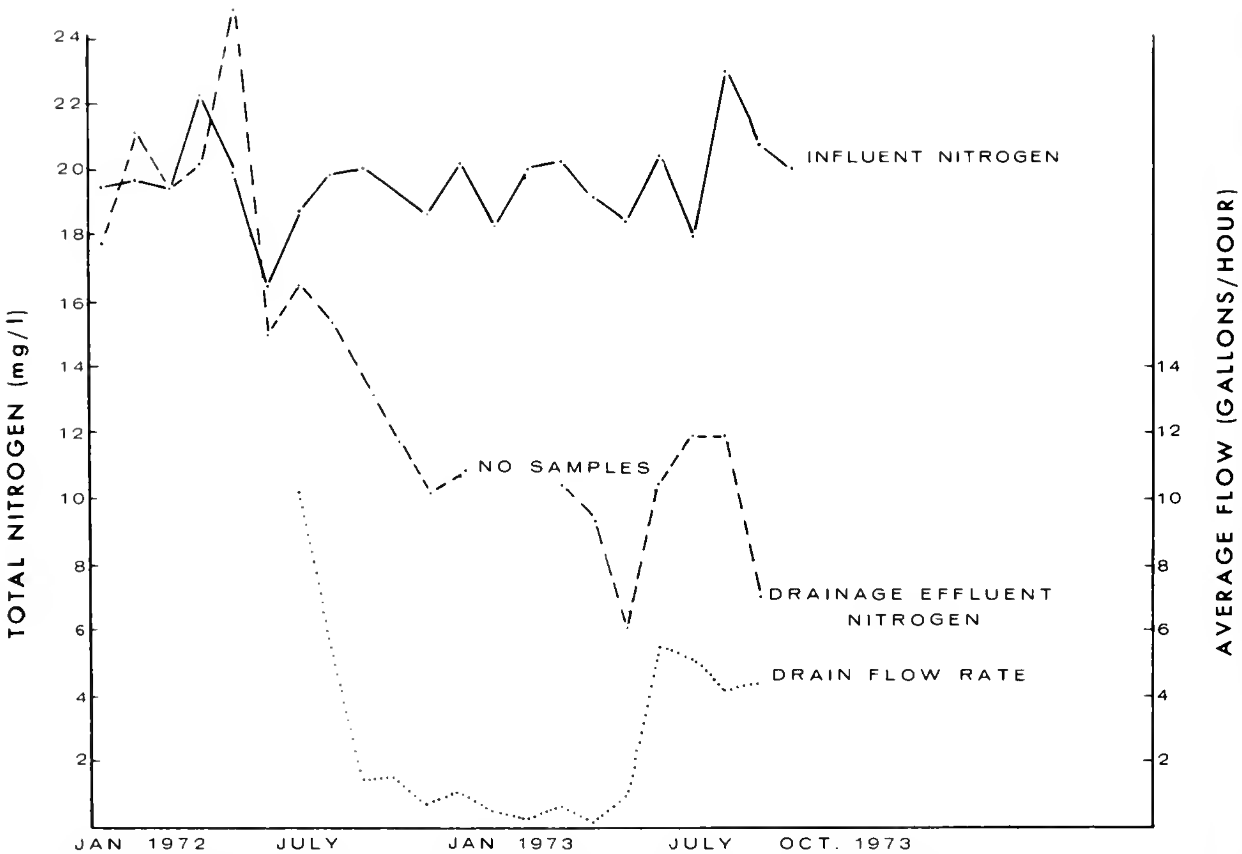


FIGURE 29 SMALL CONTROL POND, INFLUENT AND SUBSURFACE DRAINAGE EFFLUENT TOTAL NITROGEN CONCENTRATIONS, AND DRAINAGE FLOW

Analysis of the numbers of bacteria capable of denitrification in the grass ponds indicated populations on the order of 4 to 5 x 10^6, as compared to 1 x 10^6 in the control (covered) pond. Most of the bacteria were found in the sludge layer, with little seasonal variation in numbers. A laboratory study to determine the suitability of grass as a carbon source demonstrated that the rate of denitrification was the same with 150 mg/l of bermuda grass (either wet or dry), or 80 mg/l of methyl alcohol. In both cases, the nitrate-nitrogen concentrations were reduced from 20 to 0 in 9 days or less. When only 100 mg/l of bermuda grass were used there were still 3 mg/l of nitrate-nitrogen after 12 days of incubation.

Grass-Bacteria Symbiotic Process

From the previously presented data, the following observations were used to reach the conclusion that denitrification played an important role in nitrogen removal by the grass symbiotic process:

1. Nitrogen removal by the symbiotic process amounted to 75 to 150 mg/ft^2/day.

2. Assimilation of nitrogen by plant growth amounted to only 5 to 15 mg/ft^2/day.

3. There was no nitrogen removal from the surface water of a pond covered to prevent plant growth.

4. Nitrogen-laden water percolating through a soil profile showed a reduction in nitrogen content.

5. Appreciable numbers of denitrifying bacteria were present in the grass ponds; lesser numbers were present in the control pond.

6. Grass was shown to be a suitable carbon source for denitrifying bacteria.

7. Large amounts of nitrogen were removed during the period October through April when plant growth was at a minimum.

Addition to Soil Nitrogen

Inasmuch as the grass must be returned to the ponds in order to provide energy for the denitrification process, the nitrogen assimilated during the growing season becomes a nitrogen sink, and a possible future nitrogen source. Some relatively high daily ammonia-nitrogen concentrations were observed in the effluent from the small grass ponds but not from the Bennett ponds. A portion of the assimilated nitrogen could leave the ponds in this manner or, if it were released from decaying organic matter during the following growing season, could be utilized by the succeeding crop in the ammonia form. Finally, part of the assimilated nitrogen could remain in the sludge layer accumulating in the bottom of the pond. The sludge layer was investigated in an attempt to evaluate what might be happening to the nitrogen incorporated in plant tissue. Table 20 summarizes the findings.

Table 20. Nitrogen assimilated by plants and accumulated in sludge

Pond	Nitrogen					
	Plant use 1972	In sludge[c/] Jan 1973	In sludge[c/] Spring 1973	Plant use 1973	In sludge[c/] Oct 1973	In sludge[c/] Nov 1973
	lb/acre					
Small grass 1	291[a/]	111		146	246	227
2	291[a/]	89		130	214	238
3	291[a/]	62		130	216	
4	291[a/]	110		115	160	194
5	269[b/]	116		269	139	252
Control	0	0		0	0	0
Bennett West	291		281	88		292
Bennett East	152		239	47		218

a/ Production estimated at least equal to Bennett West.
b/ Production estimated at least equal to 1973.
c/ New nitrogen above the original soil quantities.

The sludge layer varied from near zero to almost 1 inch thick and was of very low density. Some of the variations in sludge nitrogen can be explained by the differences in crop production shown in table 18. Differences in sludge nitrogen between January and November 1973 are also due to the state of crop submergence and organic residue decomposition. The refractory portion of the crop residue did not decay after nearly 1 year of submergence.

Regardless of these variations, it was concluded that most of the nitrogen assimilated by the grass would remain in the sludge for more than a year. It was not possible to predict if sludge nitrogen would recycle into the pond.

Summary Nitrogen Removal Pathways

Figure 30 illustrates the distribution of nitrogen by three major pathways (plant uptake, denitrification, and addition to the soil are lumped under one category). From the previous discussion, denitrification is the major removal mechanism of that group, a conclusion supported by the fact that plant assimilation normally amounted to only 5 to 15 mg/ft^2/day. Removal by subsurface drainage, though a small portion of the total removal by the grass system, played a significant role throughout the year.

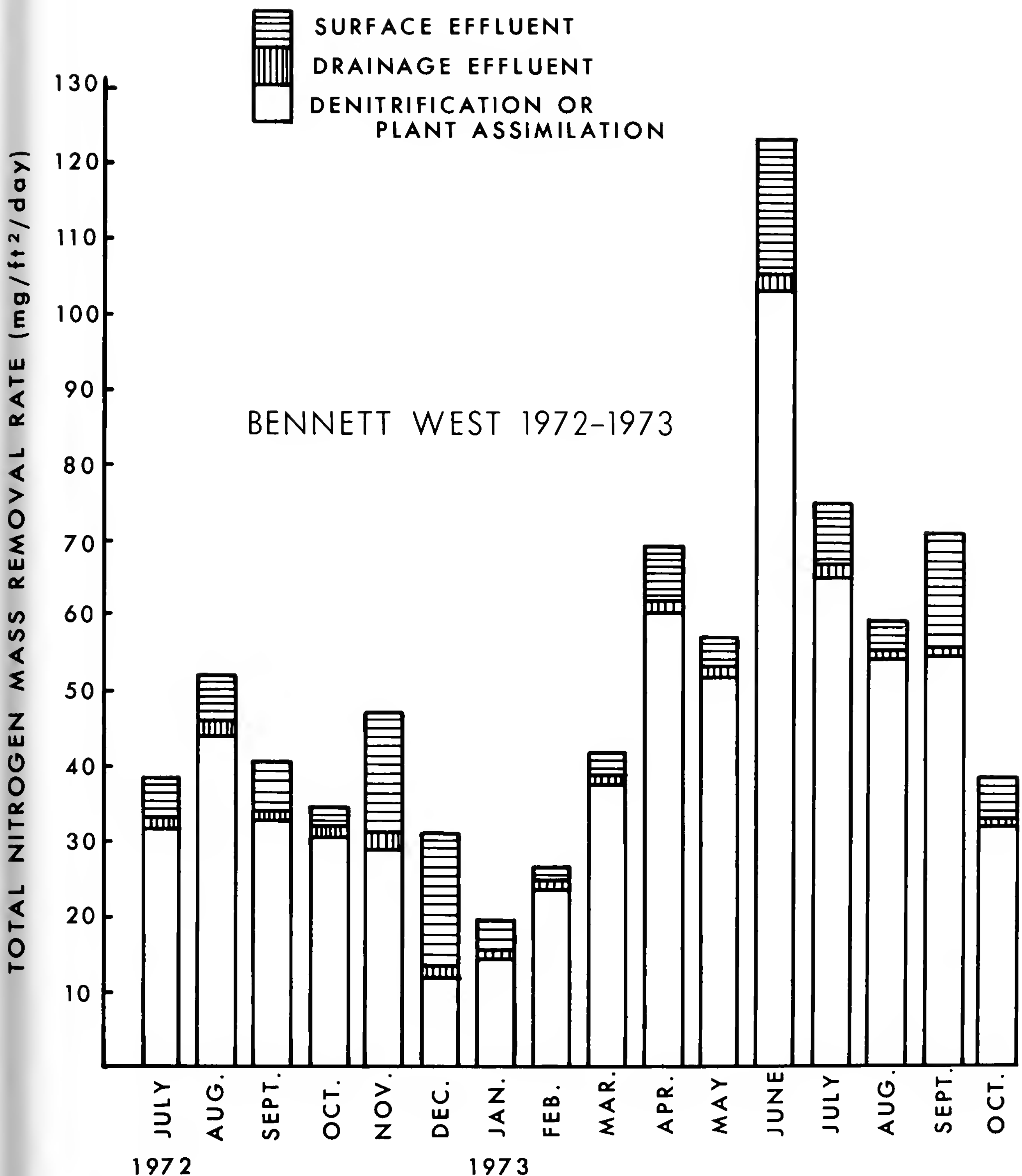


FIGURE 30 REMOVAL OF INFLUENT NITROGEN BY SURFACE EFFLUENT, DRAINAGE EFFLUENT, AND DENITRIFICATION OR PLANT ASSIMILATION

Factors Affecting Nitrogen Removal

The figures demonstrating nitrogen mass removal rates (figures 26 and 27) indicated that all the ponds had similar removals and that the most apparent factor affecting the removal was climatic condition. Other possible factors affecting the mass removal rate are depth, detention time, influent loading, and grass varieties. Operational flexibility and stability of the ponds were poor and seldom permitted segregation of each of these factors while holding all others constant.

Depth and Detention Time

Operational conditions for Bennett West and Bennett East for two periods during the study are summarized in the following tabulation, and can be used in determining the combined effect of depth and detention time.

		1973 Period			
		March		July 12-31	
Item		West	East	West	East
Influent loading	$mg/ft^2/day$	41.5	44.6	66.0	74.2
Removal	$mg/ft^2/day$	37.6	39.5	60.2	65.6
Detention time	days	4.5	8.3	4.0	6.9
Depth of water, average	inches	3	6	3	6
Water temperature average	°C	13.6	14.0	25.6	25.6
Effluent concentration	mg/l	2.03	2.92	2.95	5.93

Under the operating conditions of March 1973, water depths of 3 inches were more effective than water depths of 6 inches in nitrogen removal, and a detention time of 4.5 days was more effective than 8 days. For the July 12-31, 1973, period, the lesser depth and shorter detention time again were more effective in nitrogen removal than the 6-inch depth and the 6.9-day detention time. However, data from the total period (figure 26) point to the conclusion that neither depth of water nor detention times have important bearing on mass rates of nitrogen removal. Indications are that the shallower depth and less prolonged detention time produce a better effluent quality.

Influent Loading

To examine the effect of influent loading on nitrogen removal, data from Bennett West pond, at different periods with approximately the same detention time, are presented in the following tabulation:

Grass-Bacteria Symbiotic Process

Item		1973 period June 9-13	June 21-25	July 2-11
Influent loading	$mg/ft^2/day$	101.1	97.8	88.7
Nitrogen removal	$mg/ft^2/day$	96.0	85.2	72.1
Detention time	days	2.7	3.0	2.5
Water temperature average	°C	26.1	27.2	26.7
Effluent nitrogen concentration	mg/l	1.48	4.61	5.88

Influent loading appears to be an important factor influencing mass rates of nitrogen removal. However, it is noted with the lowest loading rate that effluent nitrogen concentrations were highest. The opposite relationship was expected and other explanations were sought. In the tabulation which follows, the detention time lag has been used for computing removal and effluent nitrogen concentrations.

Item		1973 period June 9-13	June 21-25	July 2-11
Influent loading	$mg/ft^2/day$	101.1	97.8	88.7
Nitrogen removal	$mg/ft^2/day$	88.7	82.5	78.0
Detention time	days	2.7	3.0	2.5
Water temperature average	°C	26.1	27.2	26.2
Effluent nitrogen concentration	mg/l	3.72	5.09	2.66

Computing the removal rates using the detention time lag is much more complicated. The most noticeable difference between lag and non-lag data is that using lag data the lowest influent loading produced a surface effluent of the lowest nitrogen content as was expected. Although the highest influent loading accomplished the greatest removal, the relationship between influent loading and effluent quality was not established.

Effect of Grass Variety

The general characteristics of watergrass and reed canarygrass are described under Materials and Methods. The mass rate of removal of nitrogen in the reed canarygrass pond was slightly higher than the mass rate of removal of similar sized watergrass ponds (figure 31) and was nearly the same as Bennett West (figure 26). This higher mass removal rate is significant. Subsurface drainage flow (figure 32) from the reed canarygrass pond during the 1973 May through October period was 125 percent, and from the small watergrass pond about 30

percent of the surface outflow. The surface effluent nitrogen concentrations were much higher in the reed canarygrass pond (figure 33) but both grass varieties would be about equal in treatment efficiency if both surface and subsurface effluents were blended.

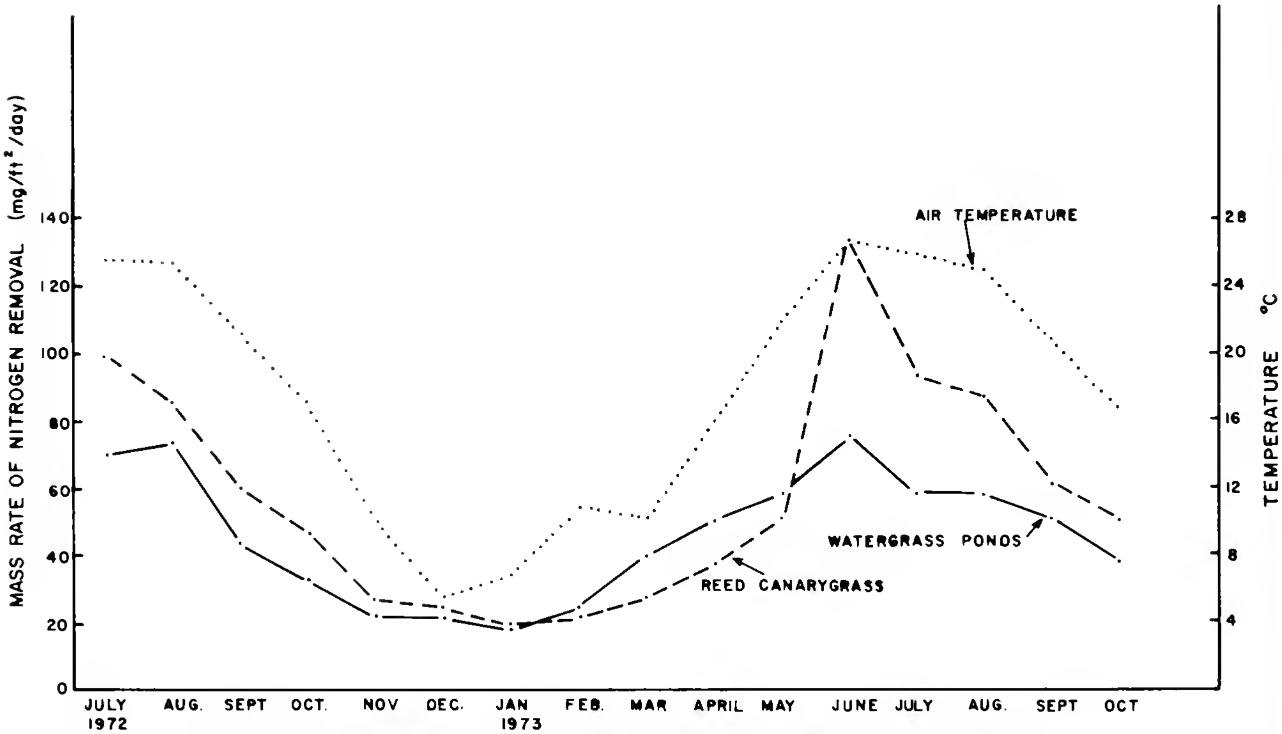


FIGURE 31 AVERAGE WATERGRASS AND REED CANARYGRASS REMOVAL RATES COMPARED TO AVERAGE AIR TEMPERATURE

This discussion points out the effect of a more permeable soil in enhancing nitrogen removal, as well as the relative unimportance of the grass variety, except in terms of the desirability of the crop produced, for enhancing rates of removal.

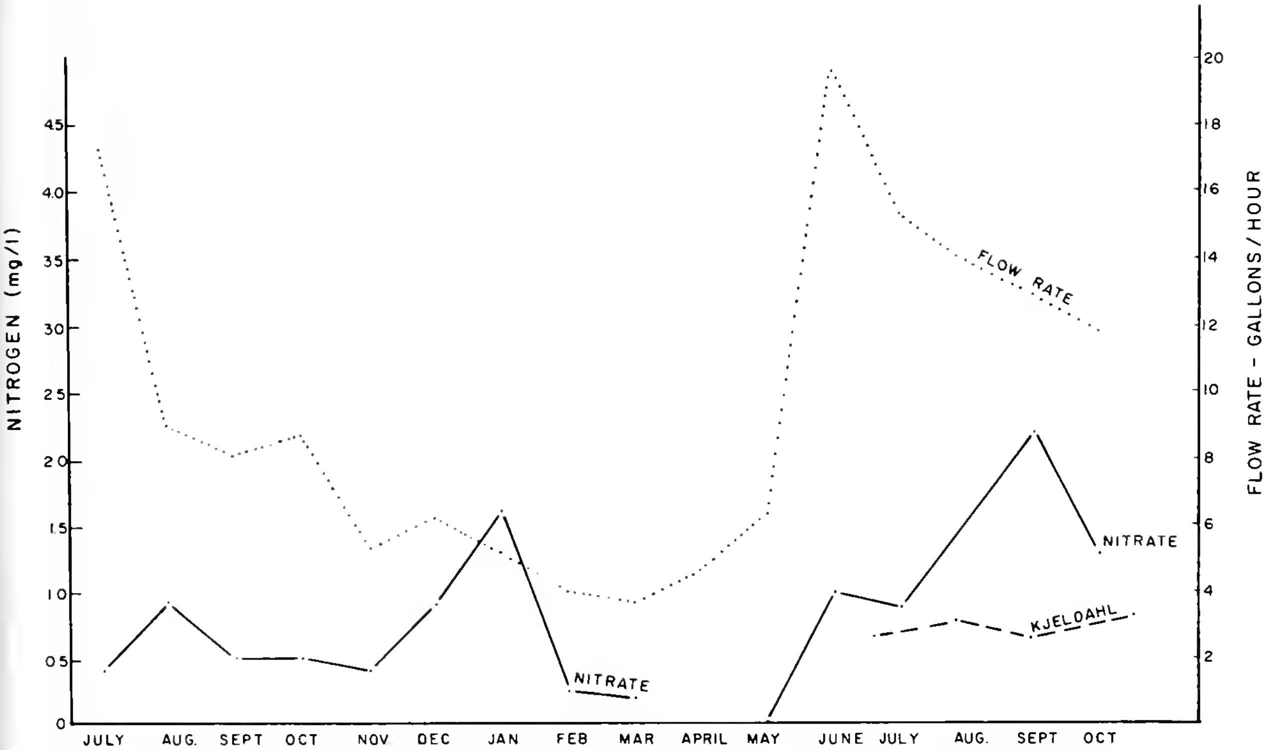


FIGURE 32 REED CANARYGRASS POND - SUBSURFACE DRAINAGE CONCENTRATION AND FLOW

Climatological Effects

The rate of nitrogen removal in the grass ponds showed a pattern similar to that observed in the algal ponds in that plots of either sunlight or temperature and nitrogen removal were roughly parallel (figure 31). Not enough of the right kind of data was collected to differentiate between the effect of incident radiation and temperature on nitrogen removal. Since denitrification played such an important role, it is likely that temperature was often the controlling factor, assuming that there was sufficient light to produce the needed organic carbon for the nitrogen removal process.

Submergence of Plant Material

Though not determined, submergence of the plant material either by waterfowl or climatic conditions could have an effect on the removal rates from enhancement for decomposition to reduction in plant growth.

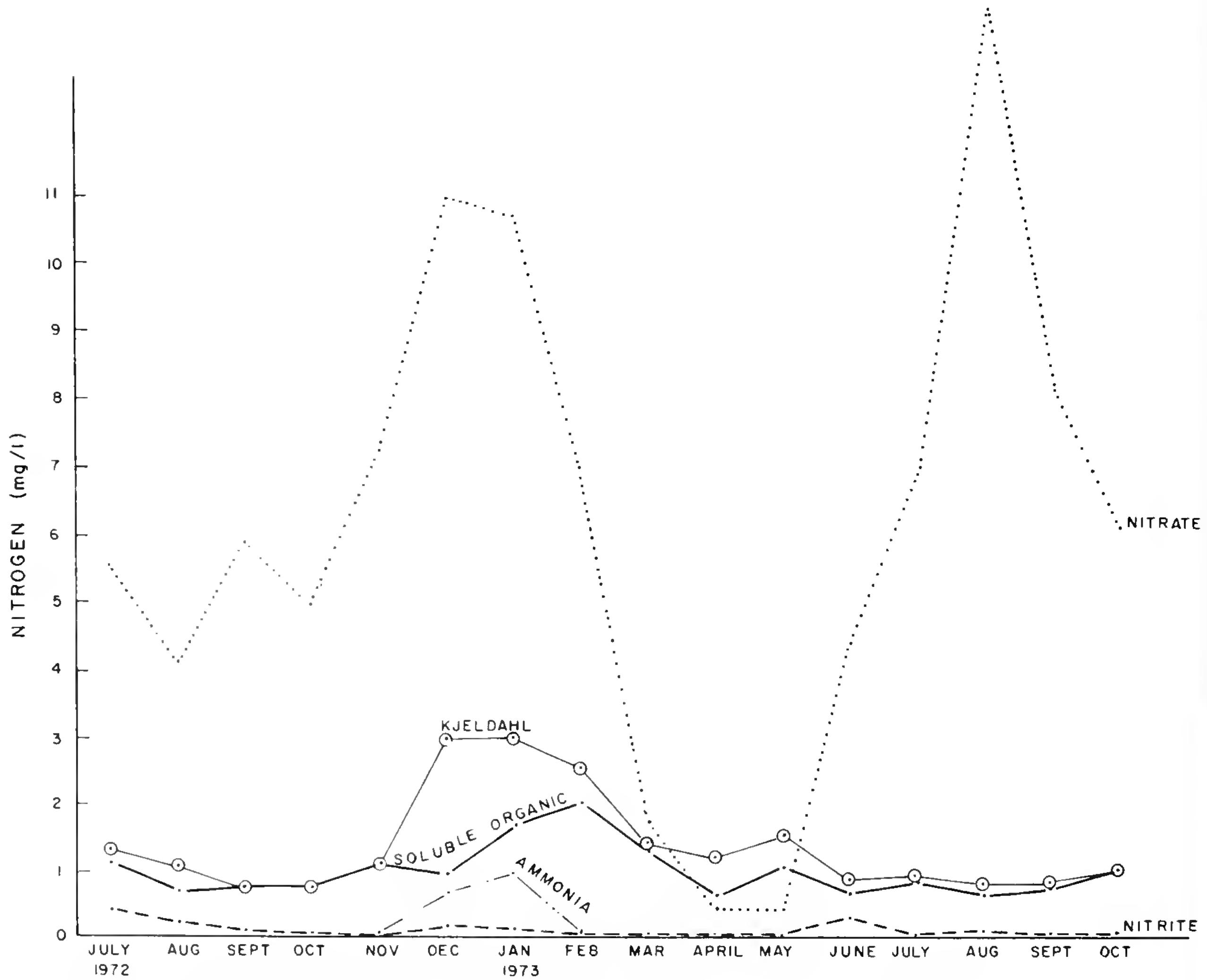


FIGURE 33 REED CANARYGRASS POND EFFLUENT-MONTHLY AVERAGE CONCENTRATIONS OF NITROGEN FORMS

Summary Factors Affecting Nitrogen Removal

The discussion on nitrogen removal has shown that most of the nitrogen left the liquid system by the process of denitrification.

Examining the data on the basis of removal from the liquid system per unit of surface area per unit of time (mass removal rate) shows that removal follows closely the curve of air temperature; however, there are departures from an exact relationship. It appears that the rate of removal can be increased by increasing the amount entering the system per unit area per unit time (load), but this will increase the concentration in the effluent. Depth of ponding and the time water was in the pond did not have a relatively important effect on the removal rate nor did the grass variety. The effluent concentration was affected by detention time or depth or both. Assimilated nitrogen accounts for a small portion of the nitrogen leaving the liquid system during the growing season and may remain in the ponds for a long time.

The influent nitrogen load exits from the ponds in surface and drainage effluents as well as by denitrification and assimilation. Figure 30 shows the mass of influent nitrogen which left the Bennett West pond by each of these exits. In most instances, denitrification accounted for the greatest amount of the influent load. The uniformity of the amount exiting via drainage reflects the constancy of the concentration and flow in the drains.

Salinity Response

Subsurface agricultural drainage water from the San Luis Service Area is estimated to contain about 7,000 mg/l total dissolved solids as drains commence operating and 3-4,000 mg/l once the major leaching is completed and an approximate equilibrium is established. As these anticipated salinity levels are relatively high for vegetative production, the response of several grass varieties to different salinities was part of the overall study. Salinity response studies were in two parts. The first consisted of salinity and plant response observations within the ponds being used for denitrification and the second part was a varietal response to different salinity levels without regard to denitrification.

Pond Salinity and Plant Succession

The seeding of the watergrass ponds in 1972 was made with seed screened from rice fields. An excellent growth of watergrass resulted in the four small ponds and throughout most of the Bennett West plot. The Bennett East plot contained some sprangletop.

In 1973, the vegetation in the small ponds consisted of about 75 percent alkali bulrush and 25 percent watergrass with most of the watergrass near the inlet. The Bennett East plot was almost 100 percent sprangletop. In the Bennett West, there was an excellent

watergrass stand in check 1 and most of check 2. In checks 3, 4, and 5 there were scattered watergrass and sprangletop and a fair stand cattails and other sedges.

The vegetative changes described probably occurred because of the salinity levels in the water and soil during germination. The specific conductance (EC in micromhos/cm) of water at the Center ranged from 2,500 in the summer to 6,500 in the winter (to convert EC to total dissolved solids, TDS, for these waters, a factor of 0.82 has been found appropriate). The influent water at the Bennett plot ranged from 2,000 to 10,000 micromhos/cm.

The wintertime evapotranspiration rate was slight while the summertime rate amounted to about 25 percent of the inflow volume. Salinity measurements at the Bennett plot confirmed this salinity increase. The salinity of the soil solution generally was much higher than the salinity of the surface water (table 21).

Table 21. Specific conductance of surface and 6-inch probe sites (1973) at the Bennett plot (micromhos/cm)

Site	1		2		3		4	
	Surface	6"	Surface	6"	Surface	6"	Surface	6"
April	3,000	6,300	5,500	7,000	3,100	7,900	7,000	7,900
July	2,000	5,000	5,000	4,200	2,200		5,500	8,000

It has been suggested (Miller, 1973) that above 5,000 mg/1 TDS (equivalent to 6,100 micromhos/cm) watergrass seedlings have difficulty in becoming established. In Bennett West check 1, the location of probe site 1, watergrass seeds germinated and a good stand was established in 1973. A fair stand was established in Bennett West check 2 and part of check 3. Very little watergrass was established in any of the Bennett East checks and vegetation was mostly from the grass identified as sprangletop. The unevenness of the land in the ponds is the cause attributed to the plant succession. The high dry areas where soil salinities were high discouraged the watergrass and led to the growth of sprangletop. In the lower wet areas, growth of cattails and other plants more tolerant of wet environmental conditions excluded the watergrass. Only in between these two extremes were conditions favorable to watergrass.

The small watergrass ponds at the treatment center were kept flooded during May and June 1973, and seed germination took place, but only a small portion of the seedlings became established.

Greatest populations of watergrass in 1973 were in the portion of the pond near the inlet; alkali bulrush plants became established in those portions of the ponds away from the inlet. Salinity measurements of surface and drainage effluent for the ponds were about 5,000 mg/l TDS in April 1973. If actual soil moisture had higher salinities, the plant succession which took place in these ponds would be explained.

Although total dissolved solids concentrations in surface and drainage effluent from the reed canarygrass pond were similar to those in the effluents from the watergrass ponds, no salinity damage was observed.

Varietal Response

To determine the response of several plant varieties to three levels of salinity; six large squares, 12 feet square, were each further divided into nine smaller areas, 4 feet square, making a total of 54 of the smaller areas. Each of the six following grasses was apportioned nine of the smaller areas for planting: watergrass, alkali bullrush, reed canarygrass, Goars fescue, coastal bermuda grass, and coast cross bermuda grass. Three small areas of each grass were grown in each level of water salinity, 5,000, 10,000, and 15,000 mg/l TDS.

First plantings made in the spring of 1972 were unsuccessful. Soon after germination, watergrass and alkali bulrush seedlings died and the other varieties, propagated by stolons, clones, etc., soon succumbed. Fatalities gave the appearance of herbicide poisoning rather than the usual salt damage.

The surface 2-3 inches were then removed from the experimental area and soil brought in from a canal bank. It was not until the summer of 1973 that plants of these six varieties became established and flooding commenced. Water was maintained at approximately 3 inches from August 28, 1973, through October 31, 1973, except for minor periods when operational difficulties occurred.

Average electrical conductivity of the applied water with extremes are given in table 22.

Table 22. Electrical conductivity of water in varietal response grass ponds

Square	Average	Range
	micromhos/cm	micromhos/cm
1 and 2	6,700	4,200 - 10,500
3 and 4	11,800	5,000 - 17,200
5 and 6	20,600	14,400 - 24,000

Notes taken on plant conditions in the various large squares follow:

Squares 1 and 2 - Approximately 5,500 mg/l TDS.

September 11. Watergrass and alkali bulrush seed had floated throughout square and where deposited in the Goars fescue areas, the resulting plants have grown and matured. New shoots are developing from the reed canarygrass clones and the Goars fescue is growing vigorously. There is not much alkali bulrush but such plants as are established are vigorous. Both bermuda grass varieties are putting out new growth and encroaching on the reed canarygrass area.

October 31. At the end of observations varieties in this level of salinity appear vigorous and healthy and with the exception of two fatalities in the Goars fescue areas, no growth inhibitions of any kind were noted.

Squares 3 and 4 - Approximately 9,700 mg/l TDS.

September 11. Bermuda grasses have not been flooded as long as others because of operational problems. All varieties are growing vigorously and no difference between plants in these squares and the plants in squares 1 and 2 can be detected.

September 24. Watergrass has matured and some seeds are sprouting. Alakli bulrush is extending shoots into adjacent areas. Although alive, the reed canarygrass is weak, particularly in two of the 4-foot areas. Goars fescue is dying in two of the three small areas but both bermuda grasses are healthy and vigorous.

October 31. At the end of observations, the watergrass had matured and new crop seeds were sprouting but seedlings did not root. The alkali bulrush was continuing to encroach on other areas and most of the Goars fescue was dead. In two of its three areas, reed canarygrass was alive but weak and in the third area this grass was sending out some new growth. One of six bermuda grass areas was brown, but in the other areas both varieties were very vigorous.

Squares 5 and 6 - Approximately 16,900 mg/l TDS.

September 11. Watergrass in this level appeared to be affected by the soil prior to flooding. It is late and may not mature. Neither alkali bulrush nor the bermuda grasses appear to be inhibited thus far but both reed canarygrass and Goars fescue have been strongly affected.

October 12. Watergrass matured but poorly. This effect may be from causes other than salinity. Alkali bulrush encroaching into other areas by stolons. There is slight expansion of reed canarygrass in the outlet square and a small amount of Goars fescue is alive. Both bermuda grasses growing and expanding in squares where other grasses are dying.

October 31. At the end of observation, the bermuda grasses exhibited no apparent damage and the alkali bulrush had expanded into areas where other grasses were dead. There was some reed canarygrass and very little Goars fescue remaining. The watergrass was about the same as on October 12.

From the salinity and varietal response observations, it was concluded that there are plant varieties that would produce several tons of organic matter per acre per year in flooded ponds where the salinity was as much as 15,000 mg/l TDS. Of the varieties observed, alkali bulrush and the bermuda grasses were the most tolerant to high salinities. With pond salinities up to 10,000 mg/l TDS, the above plants plus sprangletop and possibly reed canarygrass would produce well, especially if the latter were established at lesser salinities. In the case of salinities up to 5,000 mg/l TDS any of the varieties studied have potential, but it was observed that watergrass seedlings might fail to take root at the upper limit.

COMBINED ALGAE-GRASS SYSTEM

A minigrass pond, described in Materials and Methods, was operated using algal pond effluent as its influent source to determine the feasibility of operating a combined system of algae and grass for removal of nitrate from agricultural tile drainage. Determining the fate of algal cellular nitrogen and any effects on grass pond operation were primary goals of the experiment.

Surface area ratio of the algal pond to the grass pond was 2 to 1 and the operating depths were 12 and 4 inches, respectively, for a volume ratio of 6 to 1. Detention time of water in the algal pond varied from 4 to 12 days from summer to winter. Allowing for evaporation, the detention time in the minigrass pond varied from 1 day in the summer to 2 days in December.

Total nitrogen in the influent to the grass pond varied from 5 to 13 mg/l (figure 34) and was less than 2.5 mg/l in the effluent except for two periods. One was during the first 2 months of operation where total nitrogen was 3 to 5 mg/l and again in January when total nitrogen in the effluent was 5 to 10 mg/l. From February until June 1973, total effluent nitrogen averaged less than 2.5 mg/l.

Influent particulate organic nitrogen to the grass pond varied from less than 1 to 6 mg/l and averaged about 3 mg/l during the year (figure 35). Effluent particulate nitrogen was reduced to less than 1.0 mg/l except during startup and was not affected by variation of influent particulate or dissolved nitrogen. During startup in July and August, the grass was small and did not fully shade the water and the effluent particulate nitrogen averaged about 2.5 mg/l.

As mentioned earlier, cellular organic nitrogen decomposition forms soluble organic nitrogen, ammonia, and refractory compounds resistant to degradation. The ammonia produced can be utilized by the grass during the growing season. Ammonia produced during the dormant season in the grass ponds could show up in the effluent. However, ammonia in the grass pond effluent was usually less than 0.05 mg/l NH_3-N. Ammonia concentrations greater than 0.3 mg/l-N were usually associated with rainfall of one-third inch or more.

Probably some ammonia formed was nitrified to nitrate by nitrifying bacteria in the ponds, although plant assimilation and adsorption to clay particles are possibilities. Soluble organic nitrogen in both the grass pond influent and effluent (figure 36) ranged from 1.0 to 2.5 mg/l. Decomposition of particulate organic nitrogen did not appear to change appreciably the amount of soluble nitrogen passing through the pond.

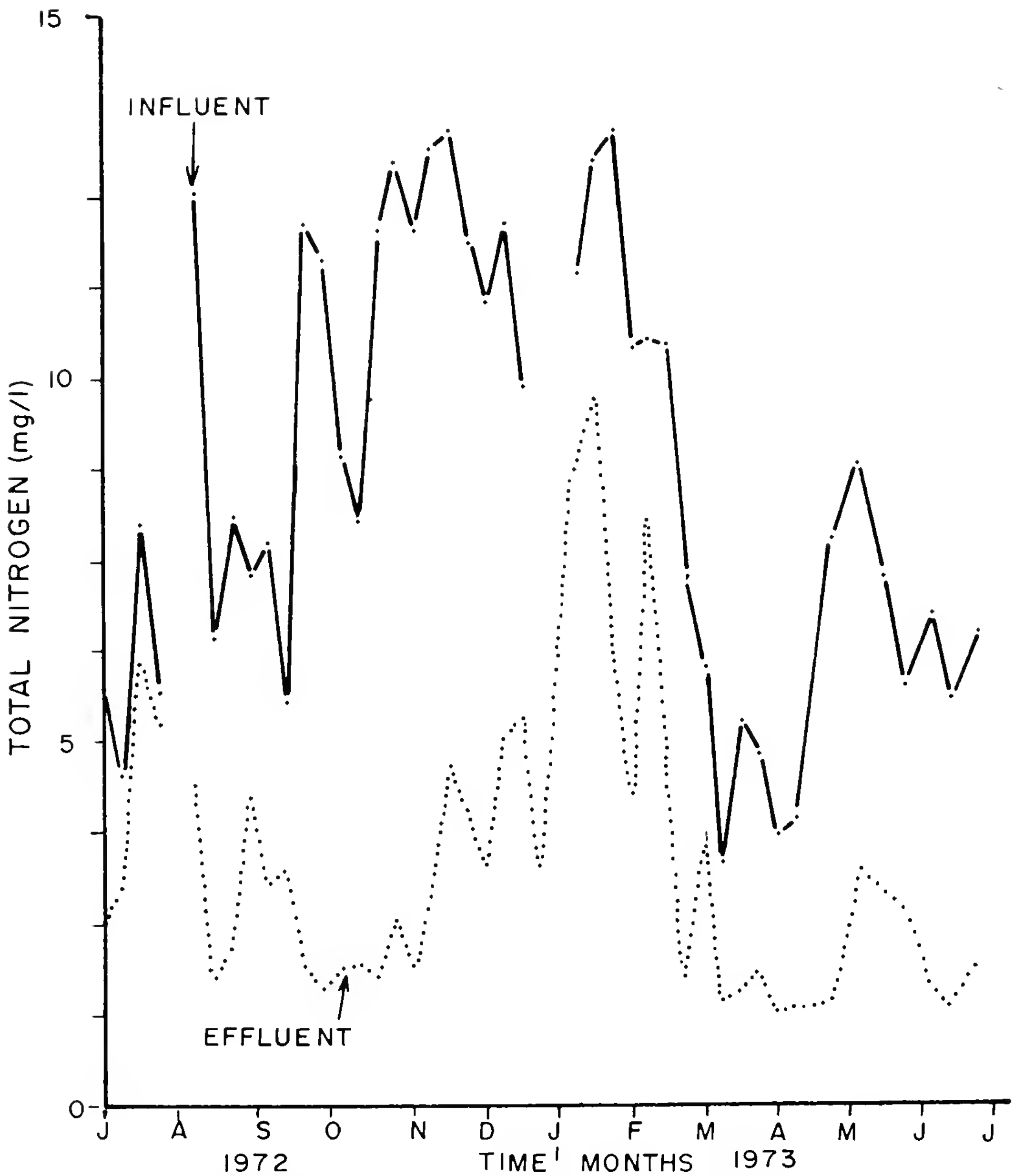


FIGURE 34 MINIGRASS POND, INFLUENT AND EFFLUENT TOTAL NITROGEN

From the results of the test, it appears grass pond operation was not adversely affected by algal cellular nitrogen. Reduction of particulate organic nitrogen from 5.0 to 1.0 mg/1 or less with 1 to 2 days detention is feasible for at least 1 year with no effect on either soluble organic or inorganic nitrogen.

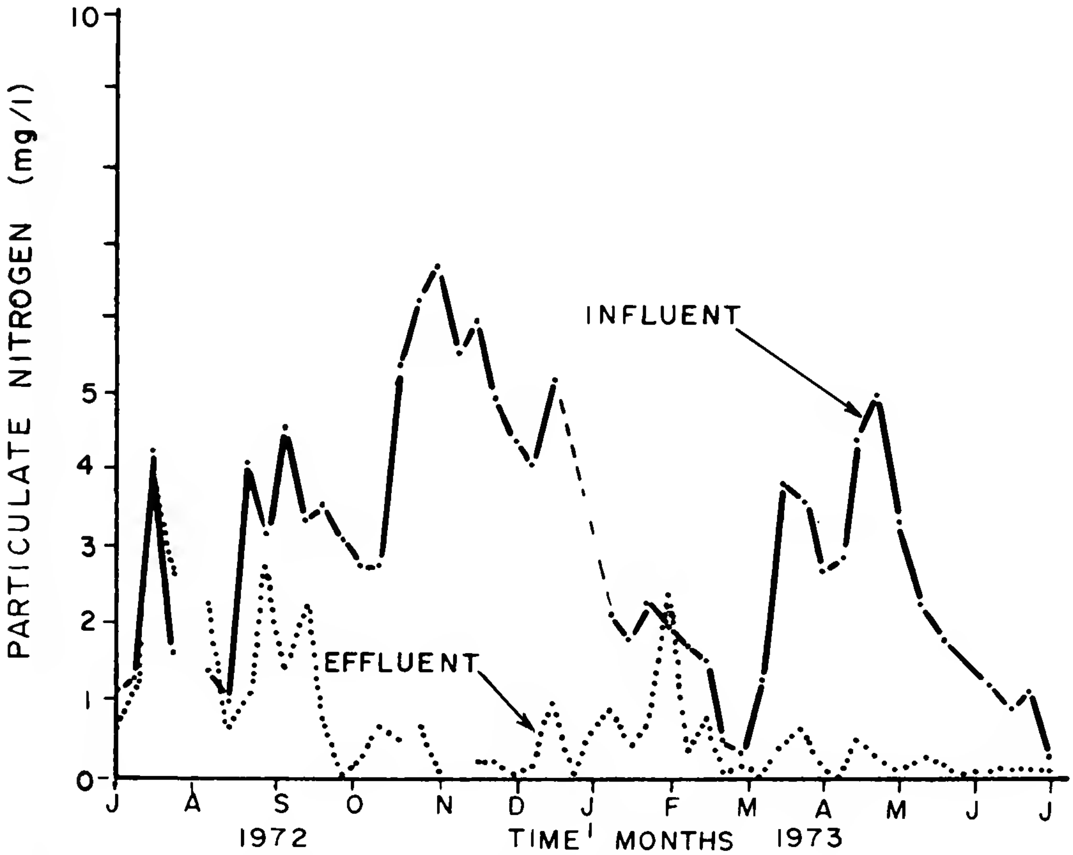


FIGURE 35 MINIGRASS POND, INFLUENT AND EFFLUENT PARTICULATE NITROGEN

Loading rates to the algal pond ranged from 60 mg/ft^2/day in February and March to 120 mg/ft^2/day in June. Total nitrogen in the grass pond effluent averaged less than 2.5 mg/1 and more than half

was soluble and particulate organic nitrogen. However, the two-to-one pond area ratio of algae to grass was arbitrarily chosen and may not have been the best combination of areas for optimum nitrogen removal.

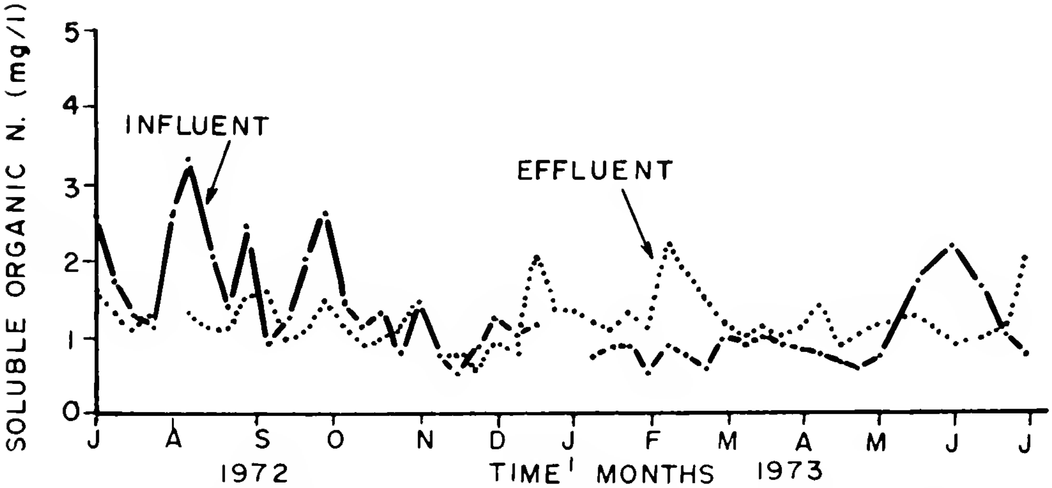


FIGURE 36 MINIGRASS POND, INFLUENT AND EFFLUENT SOLUBLE ORGANIC NITROGEN

PROCESS EVALUATION

The following section summarizes the results of the symbiotic studies in terms of process performance and estimated cost. Both performance and cost estimates are based on a limited amount of data from relatively small units. The estimates are made so that the results of the symbiotic studies can be related to those from earlier investigation of other biological nitrogen removal processes. The data from all studies can then be used to decide which, if any, of the candidate processes warrants further study on a larger scale. Such an overall comparison will be made in a forthcoming USBR-DWR joint summary of the nitrogen studies.

Effluent Nitrogen Concentrations

A general goal of the treatment studies was to produce an effluent nitrogen concentration which would not cause any significant adverse environmental effects in potential receiving waters. Based on data from the three versions of the symbiotic process, the following ranges of total effluent nitrogen concentration (maximum and minimum monthly average, mg/1) might be expected:

Algae-Bacteria	Grass-Bacteria	Combination
4 to 6	2 to 5	2 to 5

For the above estimates, it was assumed that 90 percent of the effluent algae from the algal system was harvested, and the results are based on studies conducted with an approximate 20 mg/1 influent nitrogen. In all cases, the effluent contains at least 1 mg/1 of soluble organic nitrogen which is relatively unavailable for supporting algal growth.

On a monthly average, the symbiotic process appears to be always capable of reducing 20 mg/1 to 5 to 6 mg/1 and often to 2 or 3 mg of total effluent nitrogen. These levels were achieved in the present studies for long periods in spite of operational problems which often caused large fluctuations in influent flow and nitrogen concentration.

Removal Pathways

Actual definition of the pathways involved in nitrogen removal by the symbiotic process was not achieved; however, enough inferential data are available to make some broad generalizations. The assimilation of nitrogen by grass and algae only accounted for a relatively small fraction of nitrogen removed. Laboratory tests demonstrated

that both grass and algae were suitable carbon sources for denitrifying bacteria. Tests for the activity of denitrifying bacteria were inconclusive but in general the bacteria appeared to be present in moderate numbers, with the greatest populations found at the interface between water and soil (or sludge) layer. It was concluded that bacterial denitrification played an important role (up to 50 percent removal in the algal system and 90 percent removal in the grass system) in removing nitrogen from waste water by the symbiotic process.

Cost Estimates

The following cost estimates were calculated using many of the techniques originally developed for the previous studies. The actual estimates are "appraisal grade" and were made by the SPEAD team (speedy planning, estimating, and design) of the U.S. Bureau of Reclamation Division of Design and Construction. To convert these estimates to an actual cost of constructing and operating a treatment plant specifically for treating San Joaquin Valley drainage, certain changes must be made. Some of the more important changes will be pointed out where appropriate.

Treatment Plant Acreage

The San Joaquin Valley drainage from the southern portion of the great Central Valley is expected to have an average nitrogen concentration of 20 mg/l nitrate-nitrogen. The ultimate annual flow from the San Luis service area has been predicted to be 150,000 acre-feet (48,878 million gallons). These estimates were used in the previous studies. The average monthly flow is expected to vary from 48 to 195 million gallons per day (Mgal/d) as shown in figure 37.

Algal System - The acreage required for treatment can be calculated using the data in table 23 and figures 38, for a 3-mg/l effluent dissolved nitrogen, and 39, for a 5-mg/l effluent dissolved nitrogen. The mean average monthly air temperatures (table 24) were used as a basis for determining acreage needed for treatment.

As seen in table 24, the critical treatment months are February through June when 6,000 to 9,000 acres are needed. For 7 months of the year, less than 6,000 acres are needed to achieve 3 mg/l effluent dissolved nitrogen. The pond water depth for algae should be increased to a 3-foot depth by June to prevent excessive water temperatures and algae die-off.

Table 23. Predicted San Luis Drain flow and nitrogen loading at 20 mg/l nitrogen from figure 36

Month	Flow - Mgal/d	Loading mg x 10^6/day
January	47	3,600
February	122	9,300
March	142	10,700
April	179	13,600
May	189	14,300
June	196	14,800
July	189	14,300
August	189	14,300
September	147	11,100
October	95	7,200
November	65	4,900
December	47	3,600

Table 24. Symbiotic algal system treatment area for ponds of 1-foot depth required to treat 20 mg/l nitrogen at predicted flows

Month	Average air temperature °C[a]	Nitrogen loading rate mg/ft^2/day	Treatment area (acres)[b]	Effluent dissolved nitrogen[c]
January	5.0	24	3,500	5
February	12.7	31	6,900	3
March	14.0	34	7,300	3
April	15.0	36	8,600	3
May	20.6	49	6,700	3
June	24.0	57	6,000	3
July	25.6	61	5,400	3
August	25.5	60	5,400	3
September	21.6	51	5,000	3
October	17.6	42	3,900	3
November	10.3	25	4,500	3
December	5.5	25	3,300	5

a/ Firebaugh 1972 air temperatures.
b/ 43,560 ft^2/acre.
c/ Total nitrogen would contain an additional 1 mg/l particulate nitrogen not removed by the separation process.

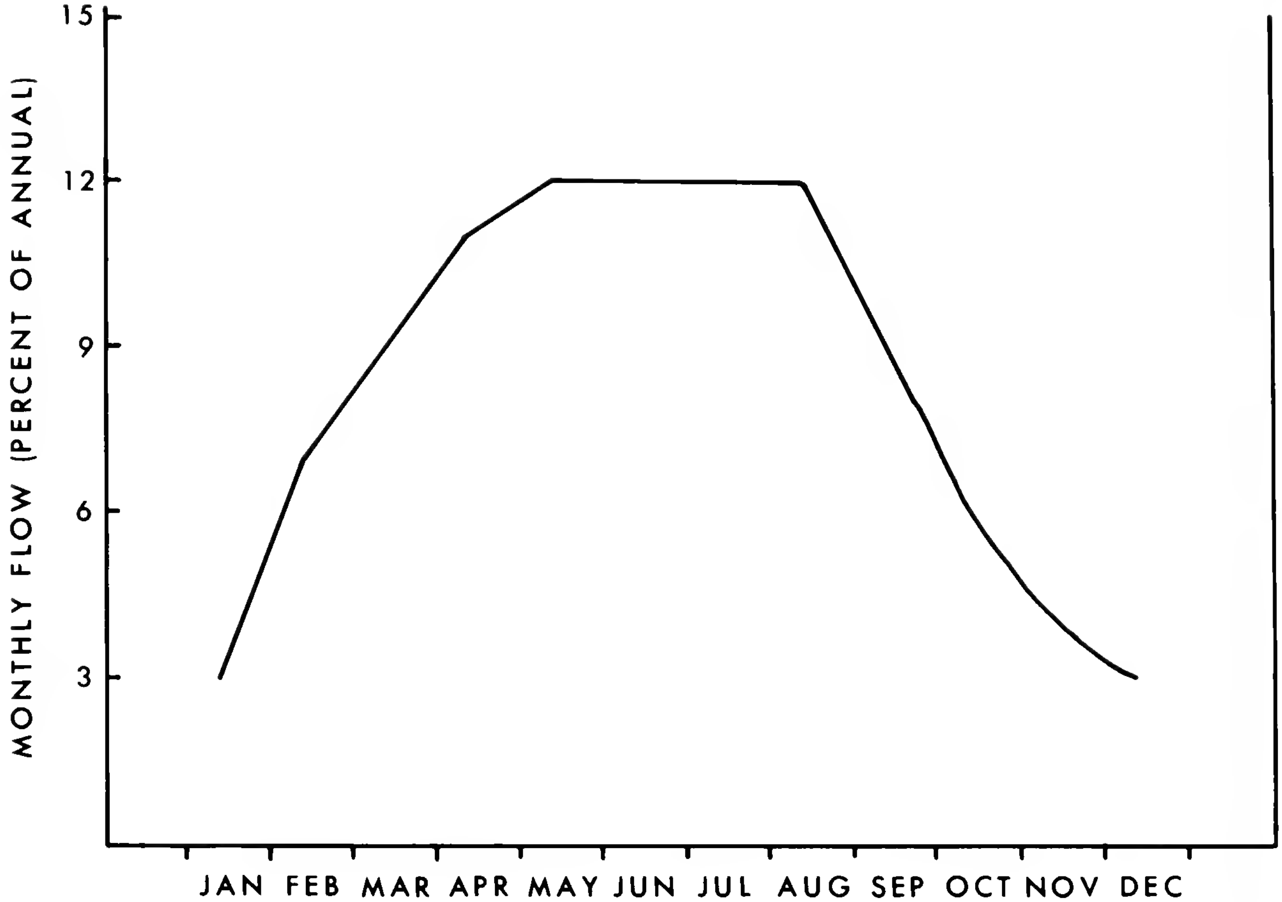


FIGURE 37 PREDICTED SEASONAL FLOW VARIATION OF THE SAN LUIS DRAIN

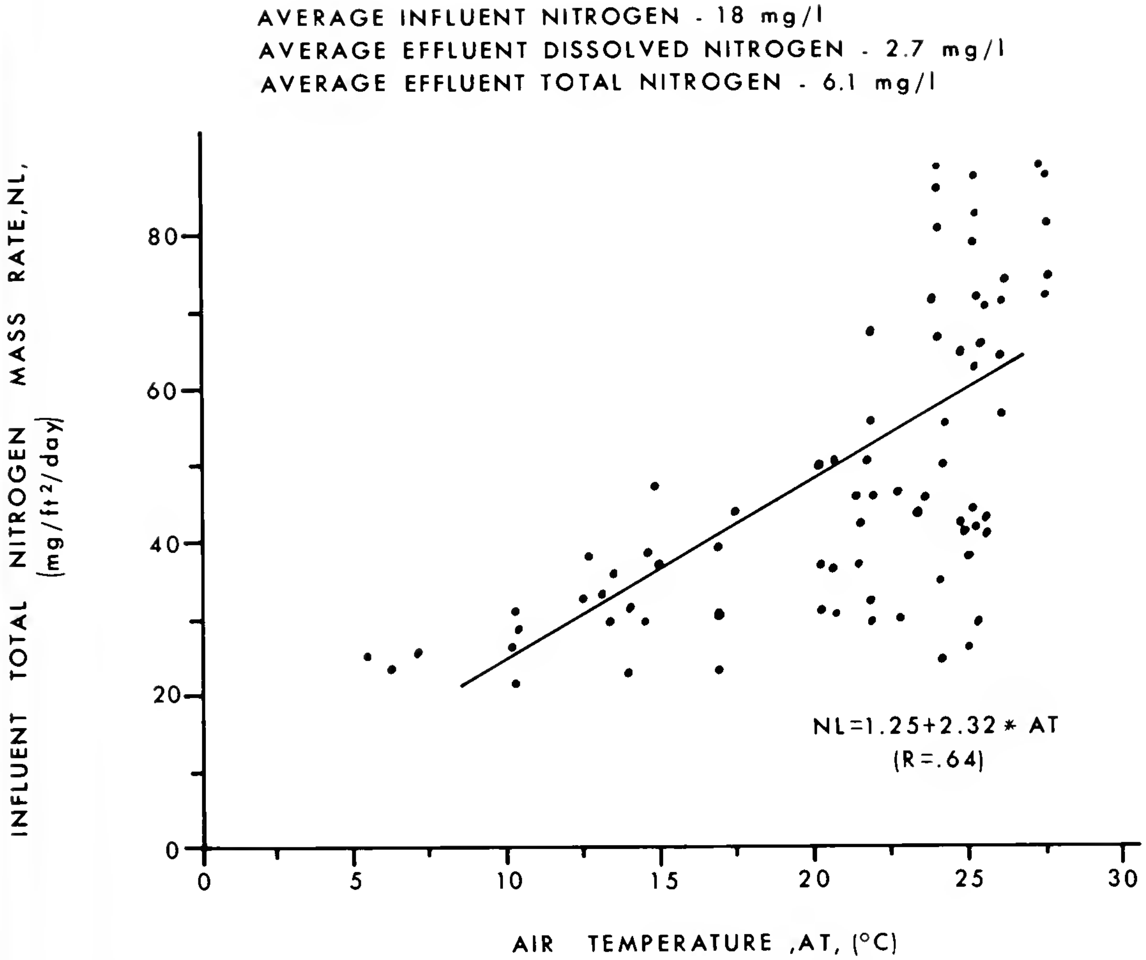


FIGURE 38 NITROGEN LOADING VS AIR TEMPERATURE FOR ALGAL PONDS OF 1-FOOT DEPTH. SELECTED POND DATA CRITERIA: LESS THAN 4mg/l DISSOLVED NITROGEN IN THE EFFLUENT

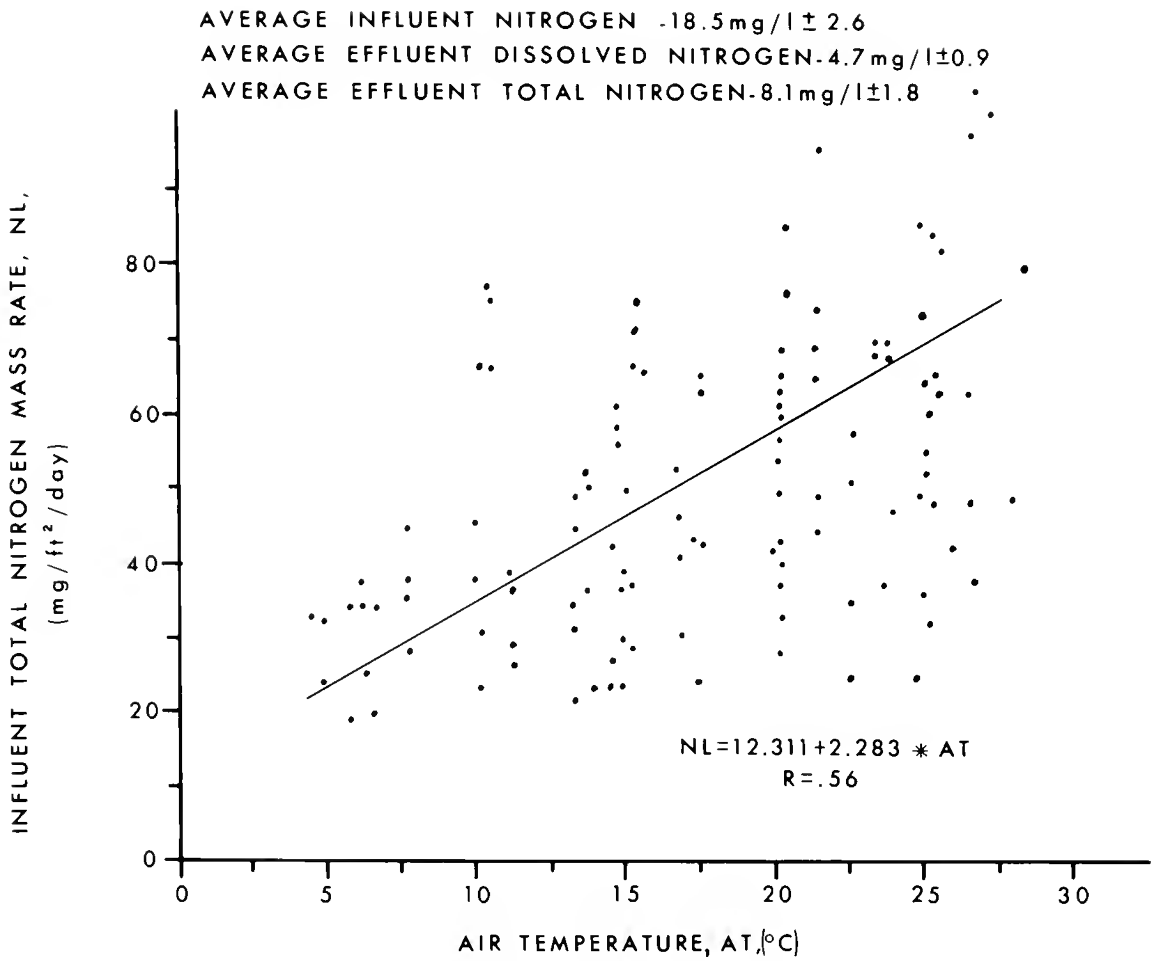


FIGURE 39 NITROGEN LOADING VS AIR TEMPERATURE FOR ALGAL PONDS OF 1-FOOT DEPTH. SELECTED POND DATA CRITERIA: 3.5 to 6.5 mg/l DISSOLVED NITROGEN IN THE EFFLUENT

Process Evaluation

Grass System - The acreage required for treatment can be calculated using tables 23 and 25 and the effluent concentration.

Table 25. Grass system, nitrogen mass removal rate (mg/ft^2/day) - monthly average

Month	Mass removal rate*
January	17
February	28
March	39
April	50
May	59
June	70
July	59
August	54
September	50
October	38
November	22
December	17

* From figure 31 for water grass.

From February 1973 through June 14, the unweighted average total nitrogen concentration of the seven ponds was 2.76 mg/l. This was during a period when monthly flows were being adjusted according to temperature records in an attempt to obtain a surface effluent with as little nitrogen as possible. Individual pond averages are shown in the tabulation which follows.

	Pond						
	1	2	3	4	5	B.W.	B.E.
Average total nitrogen - mg/l	2.41	2.19	2.39	2.81	3.74	3.03	2.75

In table 26 is tabulated the grass system acreage necessary to treat the predicted influent nitrogen load of 20 mg/l to 3 mg/l dissolved nitrogen in the effluent.

For January through August 1973, one or more of the watergrass ponds had an effluent total nitrogen concentration of less than 3 mg/l at removal rates equal to or more than those listed in table 25. In September and October, data from the different checks of the Bennett ponds have been used to project a removal rate at which 3 mg/l total

nitrogen in the effluent would be obtained. The actual concentrations for these 2 months were 3.2 and 3.5 mg/l at removal rates of 52 and 40 mg/ft^2/day, respectively.

Table 26. Symbiotic grass system treatment area to treat 20 mg/l nitrogen at predicted flows and expected effluent concentration

Month	Treatment area (acres)	Effluent dissolved nitrogen
January	4,100	3
February	6,500	3
March	5,400	3
April	5,400	3
May	4,800	3
June	4,100	3
July	4,900	3
August	5,200	3
September	4,300	3
October	3,800	3
November	4,400	3
December	4,200	3

For the months of November and December, data from 1972 were used. In these 2 months there were occasions when the effluent total nitrogen concentrations were less than 3 mg/l but the monthly averages were greater; therefore, an estimate of the removal rate was made.

Combined Algae-Grass System - Operation of a combined algae-grass system can produce a quality effluent of 2-5 mg/l total dissolved and particulate nitrogen. A system with a 2 to 1 algae pond to grass pond ratio can reach that effluent quality with a dissolved nitrogen effluent from the algal ponds of 4 to 5 mg/l. Table 27 lists the algae pond monthly treatment area necessary for an average 5 mg/l dissolved nitrogen in the effluent. A total plant of 10,050 acres with 6,700 acres of algal ponds and 3,350 acres of grass ponds would provide sufficient capacity for treatment during the critical treatment period in April.

Table 27. Symbiotic algal system treatment area required to achieve a 5 mg/l effluent dissolved nitrogen concentration in ponds of 1-foot depth

Month	Normal air temperature °C	Nitrogen loading mg/ft^2/day (from fig. 38)	Treatment area (acres)
January	5.0	24	3,500
February	12.7	41	5,100
March	14.0	44	5,600
April	15.0	47	6,700
May	20.6	59	5,500
June	24.0	67	5,100
July	25.6	71	4,600
August	25.5	71	4,700
September	21.6	62	4,100
October	17.6	52	3,100
November	10.3	36	3,200
December	5.5	25	3,300

Summary - Treatment Area - With the algal system the peak number of acres needed is in April, whereas for the grass system the peak is in February. Figure 40 demonstrates the seasonal land requirements for the systems.

The use of 20 mg/l as the influent nitrogen concentration may not be realistic for two reasons and should be modified for more definitive cost estimates. One reason is that nitrogen concentration is not expected to remain constant during the year and will affect loading. Using the annual variations in nitrogen concentration expected by the USBR as of 1971, loadings would be as shown in table 28. Comparing these estimates with those in table 23 reveals not only is maximum loading increased, but also the peak loadings are moved up to March and April instead of June when a constant influent concentration was assumed. Using the revised loading estimates, the maximum number of acres required for treatment increases to about 12,000 in April for the algal process (compared to 8,600 in April for 20 mg/l influent) and to 9,700 acres in February for the grass (compared to 6,500 in February with 20 mg/l).

The second reason why the figures may not be realistic is the possibility that there may be long-term changes in nitrogen concentration. Although Glandon (1971) predicted that nitrogen concentration (annual average) would remain relatively constant during the first 50 years of discharge of a valleywide drain, recent results

of a mathematical modeling study (Shaffer, 1974) predict that there will be significant changes in nitrogen levels in the San Luis Drain

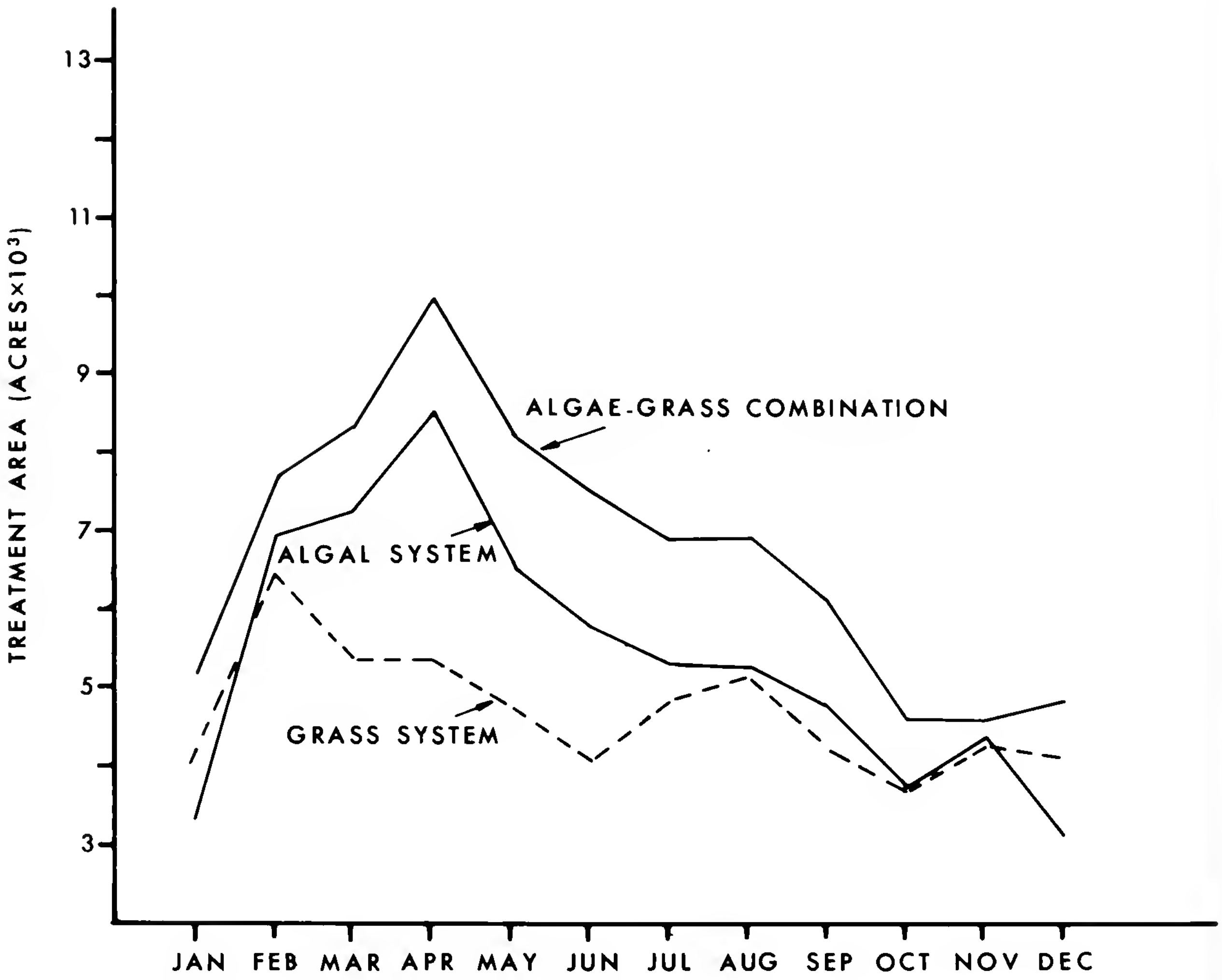


FIGURE 40 SEASONAL LAND REQUIREMENTS (BASED ON FIREBAUGH TEMPERATURES)

waters. Shaffer's predictions are plotted in figure 41. These data indicate that nitrogen levels may decrease after 30 years of leaching,

and would reach a constant level after about 50 years. These predictions are tentative and as yet have not been verified empirically but should be given some considerations in cost estimates.

Table 28. Predicted nitrogen loading from the San Luis Drain when nitrogen concentrations are varied seasonally

Month	Flow - Mgal/d	N concentration mg/l	Loading mg x 10^6/day
January	47	28	5,000
February	122	30	13,900
March	142	30	16,100
April	179	28	19,000
May	189	17	12,200
June	196	15	11,100
July	189	14	10,000
August	189	12	8,600
September	147	13	7,200
October	95	17	6,100
November	65	19	4,700
December	47	21	3,800

Treatment Plant Design - Algal System

Figure 42 is a schematic flow diagram of the symbiotic algal process.

The treatment in the symbiotic algal ponds is followed by a sedimentation process which removes 90 percent of the suspended biomass. The effluent from the sedimentation tanks leaves the plant site and the algal slurry goes to a vacuum filter for dewatering to about 20 percent solids. The effluent from the dewatering device is recycled to the influent of the sedimentation tank while the sludge is dried to between 85 and 90 percent solids by air or flash driers.

The design criteria for the major features include:

Normal operating depth	1 foot
Treatment area required at 20 mg/l influent - maximum	8,600 acres
Area per pond	160 acres
Growth chemical - Phosphorus	1 mg/l

The pond bottoms must be made impervious or underlain with tile drains to protect the ground water. The tile drains (surrounded with a gravel filter), are corrugated perforated plastic pipes placed at a 5-foot depth. The tile drains, 6-inch diameter, on a 500-foot center spacing, are tied into 8-inch collector drains which carry

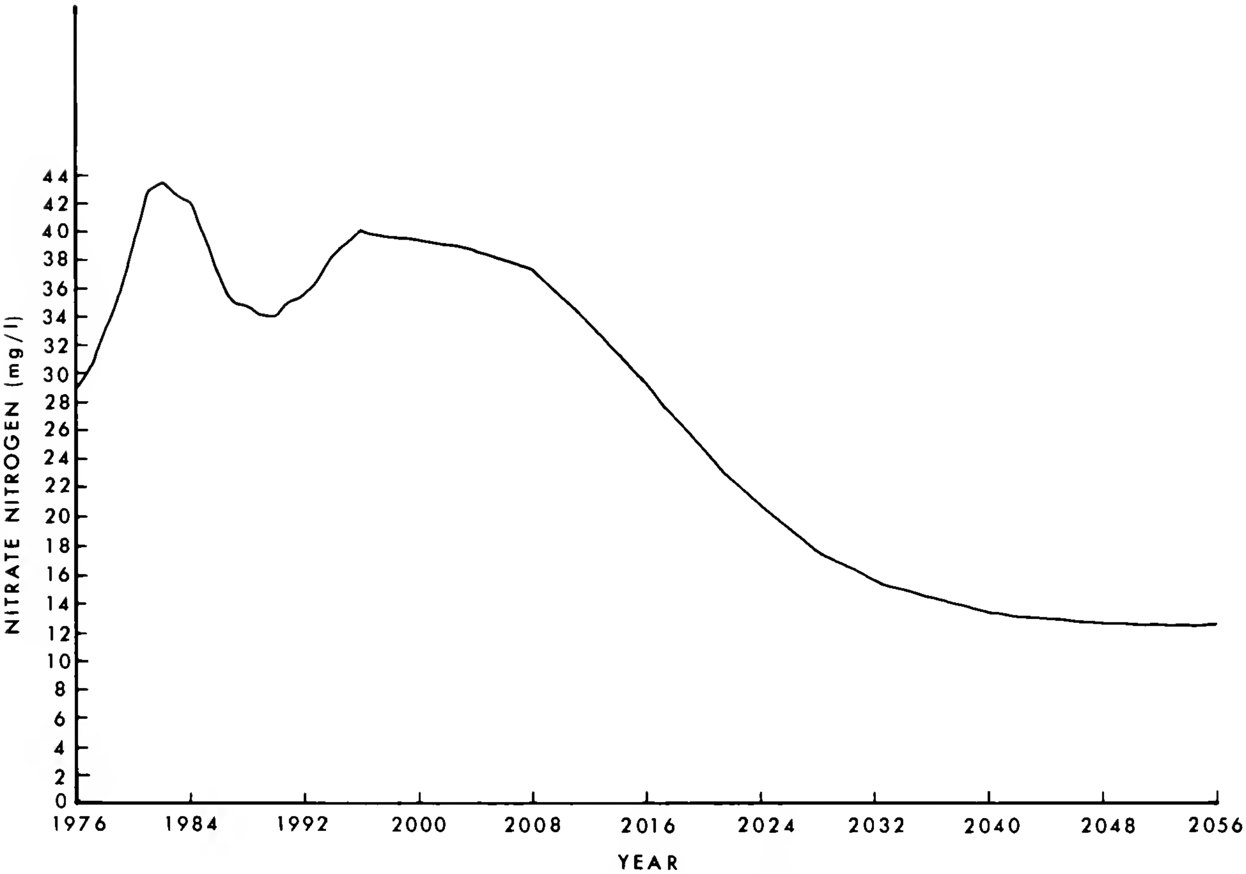


FIGURE 41 PREDICTED NITRATE - NITROGEN CONCENTRATION IN THE SAN LUIS DRAIN VS TIME (FROM SHAFFER, 1974)

the drainage water to pump sumps. The sumps are equipped with 3-hp pumps with a total pumping head of approximately 16 feet which pump the drainage effluent to a return ditch where it is mixed with the surface effluent. Preliminary estimates indicate that the tile

drainage is cheaper than an impervious bottom lining and would have the side benefit of providing additional treatment as the water percolates through the soil and a possible reduction in the treatment area required.

Exterior levees are accessible by motor vehicle and interior levees are accessible by foot. Four interior levees or cross checks are used to give a length-to-width ratio of 5 to 1. These checks divide the 160-acre pond into five strips approximately 500 feet in width and 2,600 feet long. The cross checks contain rice box-type structures that can be adjusted with flashboards for depth control. Pond depths are 4-1/2 feet, which allow a 3-foot operating depth and 1-1/2-foot freeboard. All slopes are compacted and have a slope of 2 to 1 so they can be seeded to prevent wave erosion. Concrete-lined supply ditches for each pond have a concrete check and turnout structure to control influent into the pond.

The algae harvesting methods and designs are the same as presented in Brown's (April 1971) report. Included in the design was an inline sedimentation tank to remove excess suspended material. The surface loading on the flocculation-sedimentation tank in the separation plant is considered to be 900 gal/day/ft^2, with 77 mg/l $Ca(OH)_2$ as a chemical addition. Of the three flocculants studied in the symbiotic process, $Ca(OH)_2$ was the least expensive. Loading on the vacuum filter is 0.2 gal/min/ft^2. One-half of the sludge from the vacuum filter goes to 7,000 pounds H_2O/hr driers, and the remaining half is air dried.

<u>Treatment Plant Design - Grass System</u>

Figure 43 is the flow diagram for a grass growth pond. The main design criteria of the grass ponds include:

Normal operating depth	0.5 foot
Treatment area required - maximum (20 mg/l influent)	6,500 acres
Area per pond	160 acres

The grass ponds are similar to the algal ponds but are underlain by tile drains. The grass ponds require an additional cost for seeding, but do not require any separation of suspended solids from the effluent water.

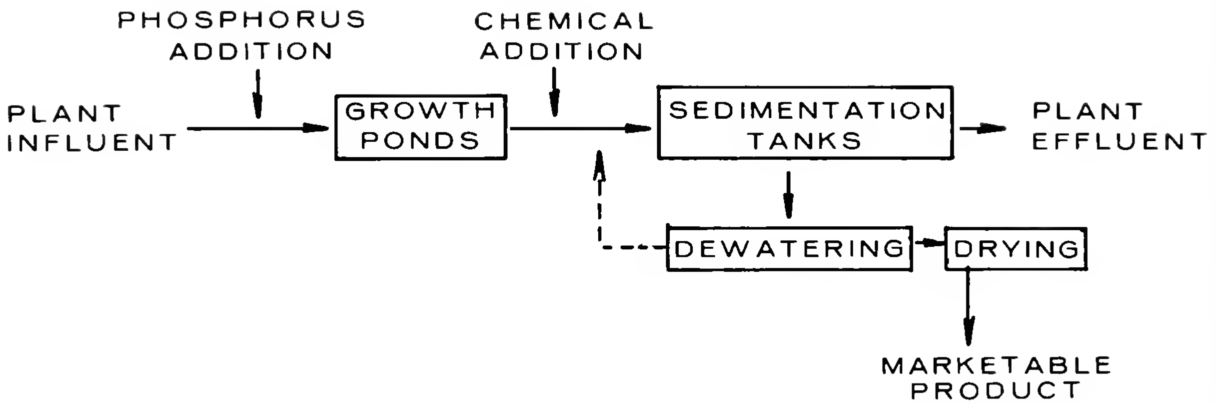


FIGURE 42 SCHEMATIC FLOW DIAGRAM OF THE SYMBIOTIC ALGAL PROCESS

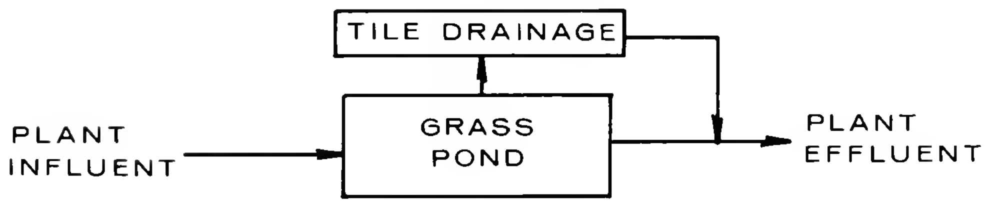


FIGURE 43 SCHEMATIC FLOW DIAGRAM OF THE SYMBIOTIC GRASS PROCESS

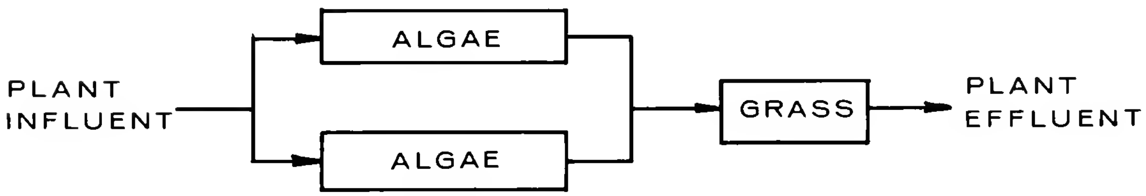


FIGURE 44 SYMBIOTIC ALGAE-GRASS PROCESS FLOW DIAGRAM

Process Evaluation

Treatment Plant Design - Algae-Grass Combination

Figure 44 is a flow diagram of the combination system. The design of this system combines two 160-acre algae ponds (without any algae harvesting) with one 160-acre grass pond in series. The final effluent from this system based on April as the critical month would contain 1 to 2 mg/l dissolved nitrogen and 1 mg/l particulate nitrogen.

Costs

Cost estimates include these factors:

1. The costs are based on January 1972 dollars.

2. The investment costs were calculated for a period of 25 years for each capital outlay debt at 5 percent interest rate. Realistically higher rates would be more appropriate; however, all previous cost estimates for the agricultural waste-water treatment studies had used 5 percent.

3. The plant will be built in five equal stages based on the buildup in quantity of drain flow. From the estimation of the drain buildup, the total period for expenditure of capital outlays will be 42 years (figure 45).

4. Costs per million gallons ($/Mgal) treated were calculated by dividing the total present worth of capital costs plus the annual cost by the present worth of the annual drain flows (5 percent interest rate).

5. Costs include engineering and contingencies.

6. The land required for a full capacity plant is to be purchased at the beginning of the project at $500 per acre.

7. Also included in the capital costs are amounts for:

   a. Fencing of the total site
   b. Lab and office buildings
   c. Maintenance and storage buildings and yards

8. Annual operation and maintenance costs include amounts for:

   a. Chemicals
   b. Electricity
   c. Personnel and equipment

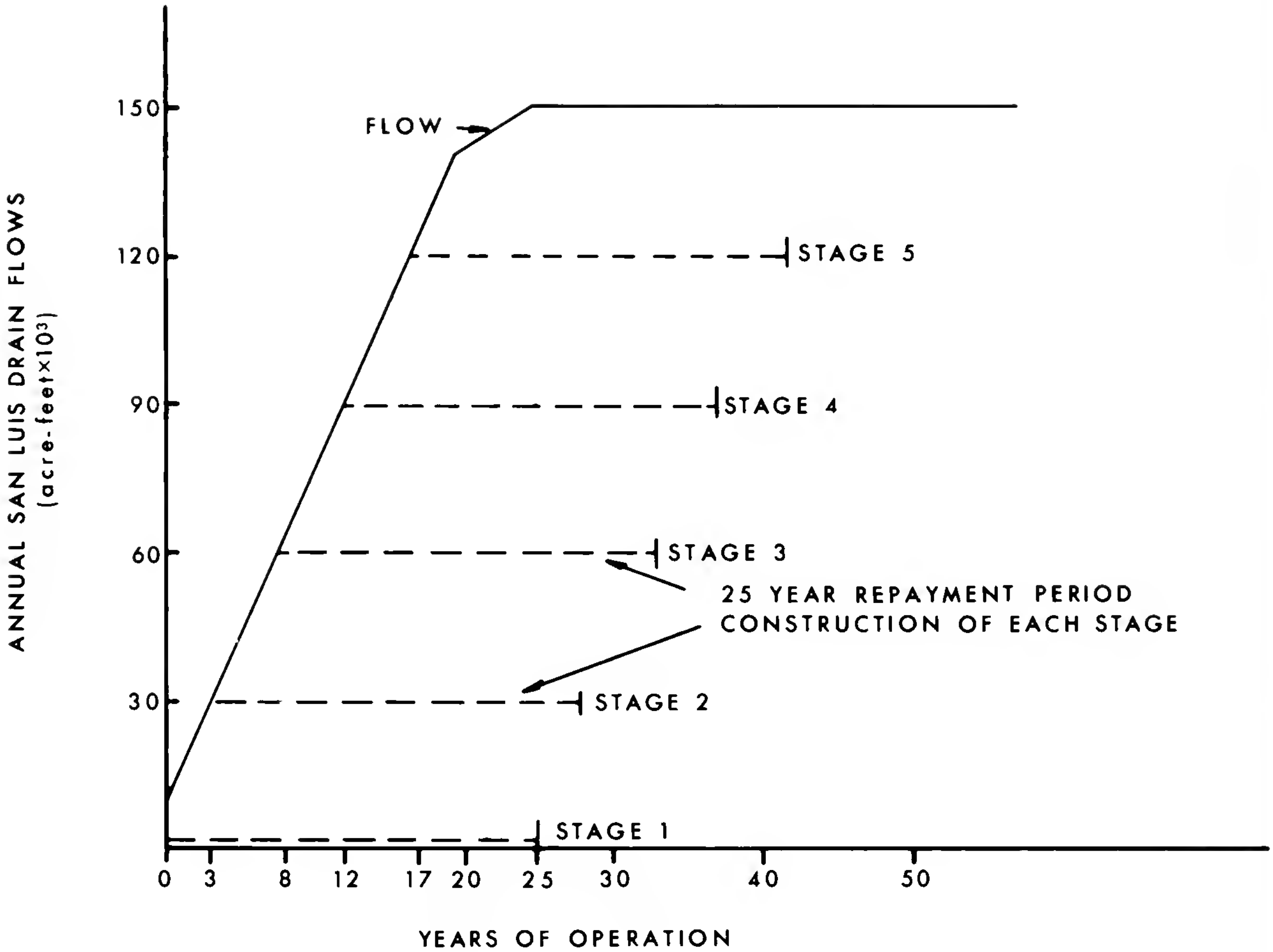


FIGURE 45 STAGE CONSTRUCTION BASED ON DRAIN FLOW BUILDUP

Capital Costs - Listed in table 29 are the capital costs per stage of the three systems. These total stage capital costs, expressed as present (1972) dollars, are:

Algae	$21,910,000
Grass	9,620,000
Combination	13,940,000

The annual buildup of drain flows expressed as present flow is 552,000 Mgal. By dividing this adjusted flow into the present worth of capital costs, capital costs per million gallons are derived for:

Algae	$39.69
Grass	17.43
Combination	25.25

Table 29. Total capital costs per construction stage in dollars*

Item	Algae	Grass	Combination
Land and fencing	4,880,000	3,670,000	5,660,000
Growth pond and distribution system	1,970,000	1,530,000	2,320,000
Separation facilities	1,960,000		
Buildings and yards	500,000	500,000	500,000
Total Stage 1	9,310,000	5,700,000	8,480,000
Growth pond and distribution system	1,970,000	1,530,000	2,320,000
Separation facilities	2,140,000		
Total Stage 2	4,110,000	1,530,000	2,320,000
Growth pond and distribution system	1,970,000	1,530,000	2,320,000
Separation facilities	1,960,000		
Total Stages 3 or 5	3,930,000	1,530,000	2,320,000
Growth pond and distribution system	1,970,000	1,530,000	2,320,000
Separation facilities	2,140,000		
Buildings and yards	40,000	40,000	40,000
Total Stage 4	4,150,000	1,570,000	2,360,000
TOTAL ALL 5 STAGES	25,430,000	11,860,000	17,800,000

* Rounded to the next 10,000

Operation and Maintenance Costs - Table 30 lists the annual O&M costs for the three systems.

Table 30. Annual costs per million gallons ($/Mgal)

Item	Algae	Grass	Combination
Growth chemicals	1.42		1.42
Separation chemicals	3.25		
Separation operation	25.16		
General operation and maintenance	11.00	11.00	11.00
Total	40.83	11.00	12.42

Total Cost of Treatment - Listed in table 31 are the total costs for treatment as derived from the designs and cost estimates. Byproduct income, if any, from the sale of harvested grass or algae would reduce these operating costs.

Table 31. Total treatment costs per million gallons ($/Mgal)

Item	Algae	Grass	Combination
Capital	39.69	17.43	25.25
Annual	40.83	11.00	12.42
Total	80.52	28.43	37.67

These treatment costs are for a 20 mg/l total nitrogen influent concentration. It may be possible to extrapolate the capital cost on a ratio basis for other influent nitrogen concentrations. For example, the estimation of the monthly nitrogen variation from the annual average of the San Joaquin Master Drain for April is 28 mg/l and February is 30 mg/l, which could increase the capital costs to:

Algae - (28/20) 39.69 = $55.57/Mgal
Grass - (30/20) 17.43 = 26.15/Mgal

Additional Benefits

A treatment system utilizing grass or algal ponds singly or in combination will create a large acreage of highly eutrophic shallow lakes. These lakes should prove a great attraction to waterfowl and in time acquire a population of fish and other water animals. Management solely for enhancement of nitrogen removal probably could create conditions that at some time of the year would be intolerable for water-inhabiting animals as well as waterfowl. However,

management to enhance waterfowl and fish habitat may provide substantial benefits with little or no adverse effect on nitrogen removal. Care will have to be taken to insure that the ponds do not become problem areas for waterfowl, especially with respect to the prevention of botulism outbreaks. The recommendations by Hunter, et al., (1969) will be implemented to lessen the possibility of botulism.

Promotion of wildlife and aquiculture can head in two directions, either to promote fish and wildlife for recreation or the promotion of commercial aquiculture for growing and harvesting of catfish and possibly crustaceans such as crayfish and freshwater shrimp.

The culturing of fish and crustaceans or other water animals for profit should be investigated in a research study to observe the effects on nitrogen removal and the potential production of a marketable product.

A further use of the subsurface agriculture drainage, prior to nitrogen removal treatment, may have a beneficial effect on the treatment process. This use would be as cooling water for nuclear energy production. The increased temperature of the water by the cooling process could enhance the nitrogen removal in the winter months.

The grass ponds could have a potential marketable byproduct in the form of hay or pasture while the algal ponds have a possible marketable byproduct, the algal biomass, which could be harvested and dried as a high-protein concentrate with uses similar to those for soybeans.

The algal and grass ponds in combination would provide system backup in the instance that either grass or algae were not functioning properly.

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